KOMP2…A translational scientific resource to catalyze biomedical research and accelerate precision medicine

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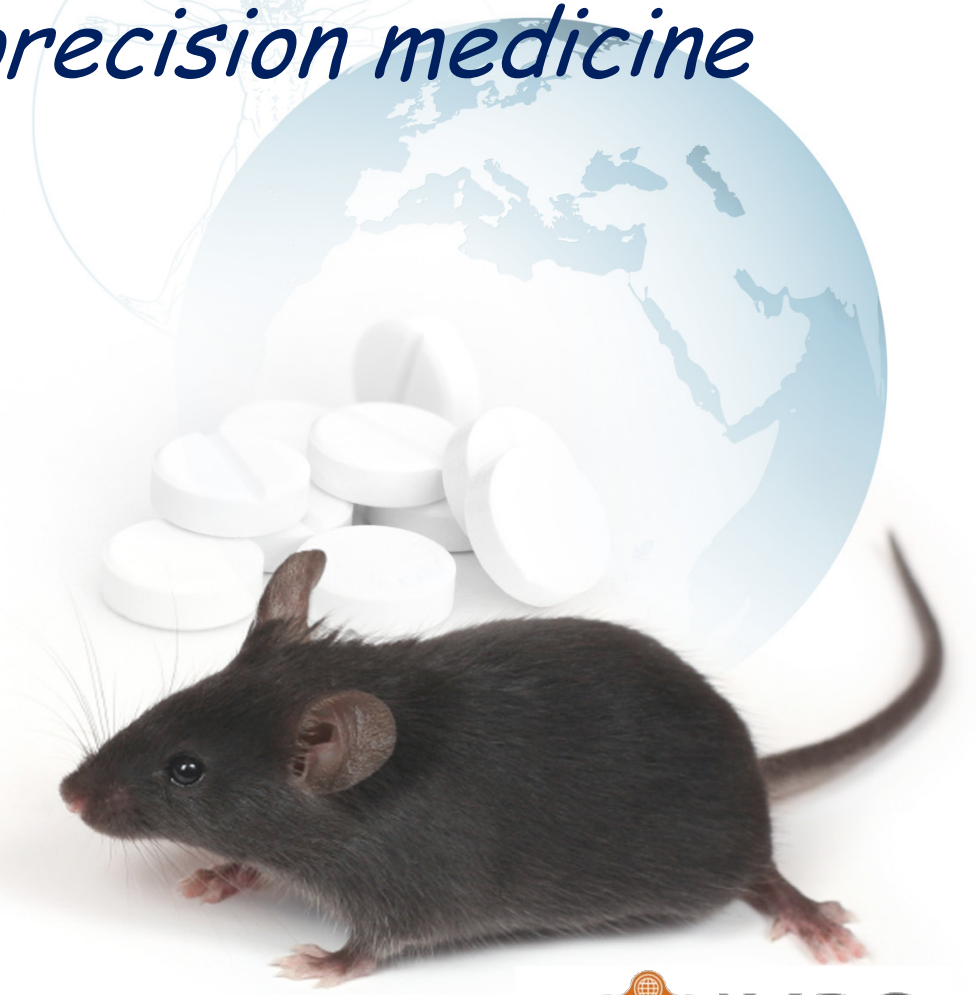
NIH Council of Councils
May 26, 2017



IMPC

KOMP2 Participants: Production & Phenotyping

Project Centers

Project Components

BCM · Sanger · Harwell

Baylor College of Medicine

MRC Harwell

PI/PD: Arthur Beaudet
Steve Brown

The Jackson Laboratory

PI/PD: Robert Braun
Steve Murray
Karen Svenson

DTCC
DAVIS · TORONTO · CHARLES RIVER · CHORI
CONSORTIUM

UCDAVIS MBP

TCP

PI/PD: Kent Lloyd
Colin McKerlie

KOMP2 Participants: Data

Centers

MPI2
Mouse Phenotyping Informatics Infrastructure

Components

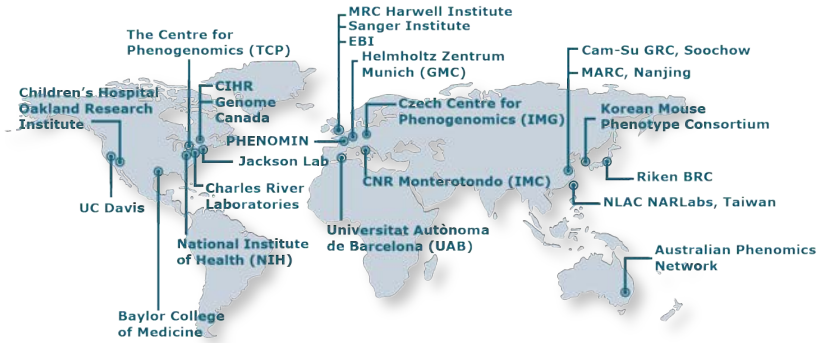
EMBL-EBI

wellcome trust sanger institute

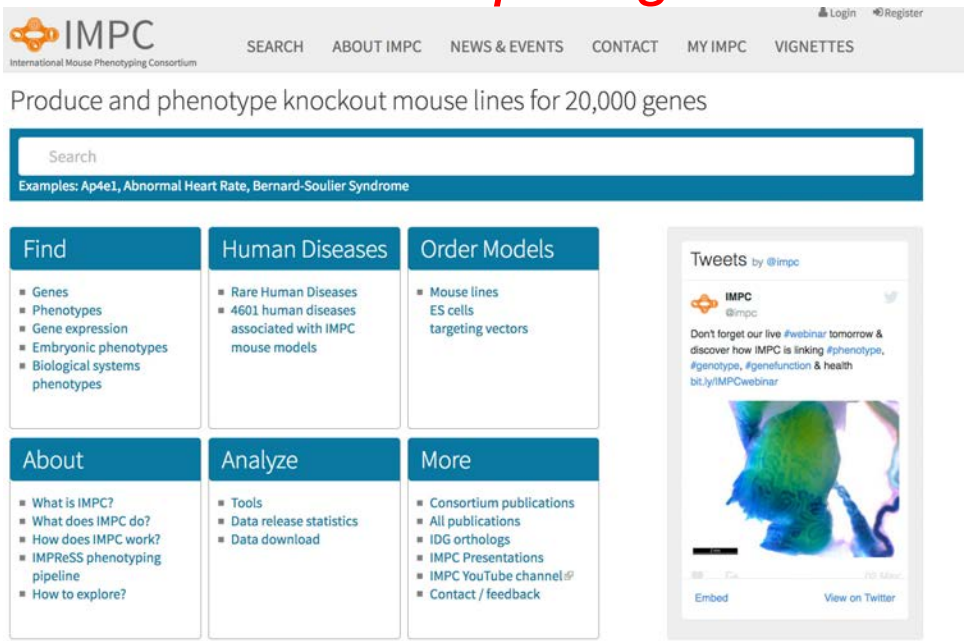
MRC HARWELL

PI/PD: Paul Flicek

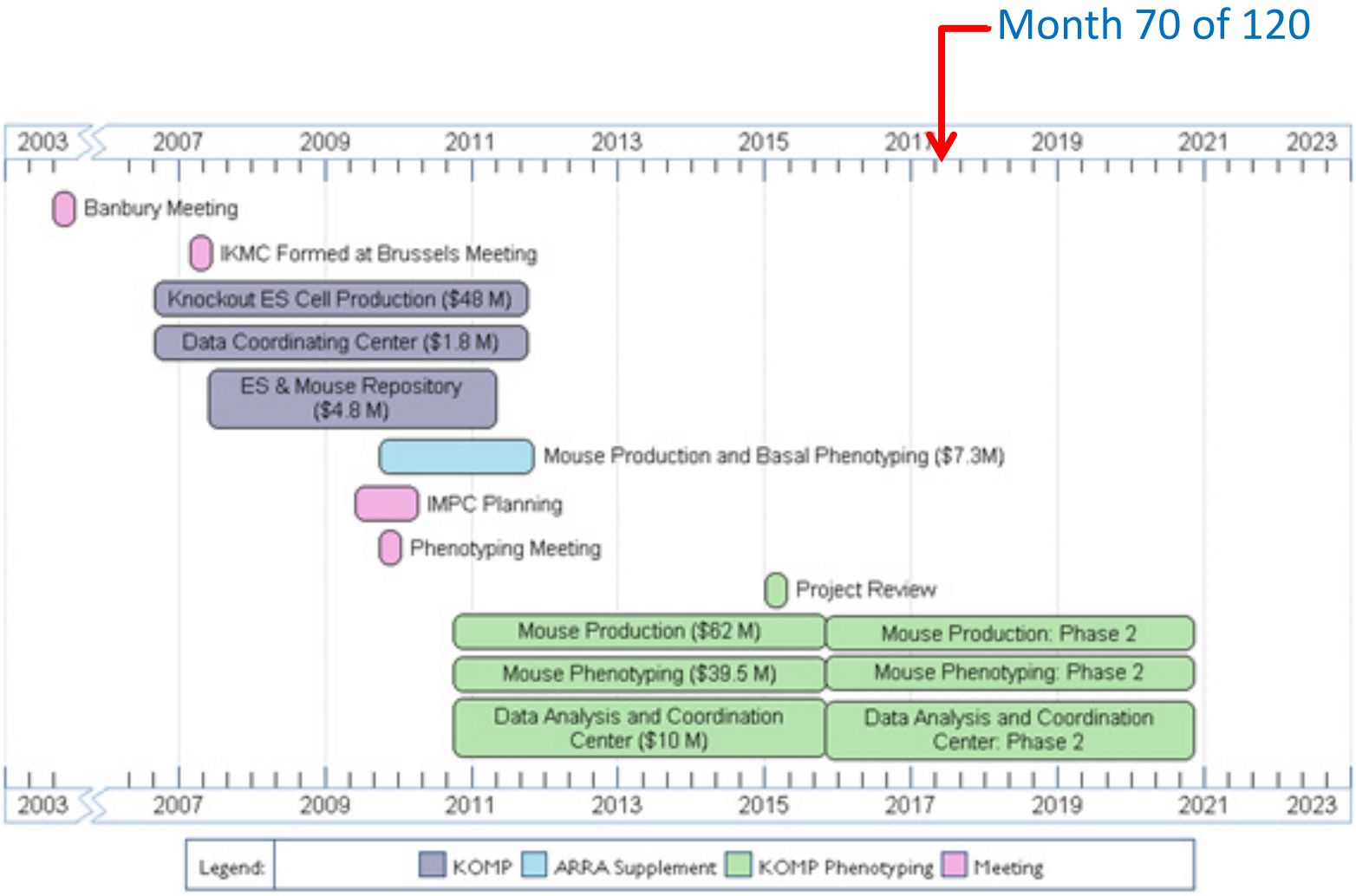
IMPC
International Mouse Phenotyping Consortium



www.impc.org



KOMP2 Project Timeline



Last time at Council…2014

- <2,000 lines produced from ESC
- ~100 lines phenotyped
- 30% embryo lethal
- Coordination within IMPC
- Website/data portal launched
- 1,237 orders received
- Few dozen publications
- Piloting CRISPR/Cas9

KOMP²

Knockout Mouse Production & Phenotyping:

a The NIH Common Fund *project*

What we've done…Where we're going

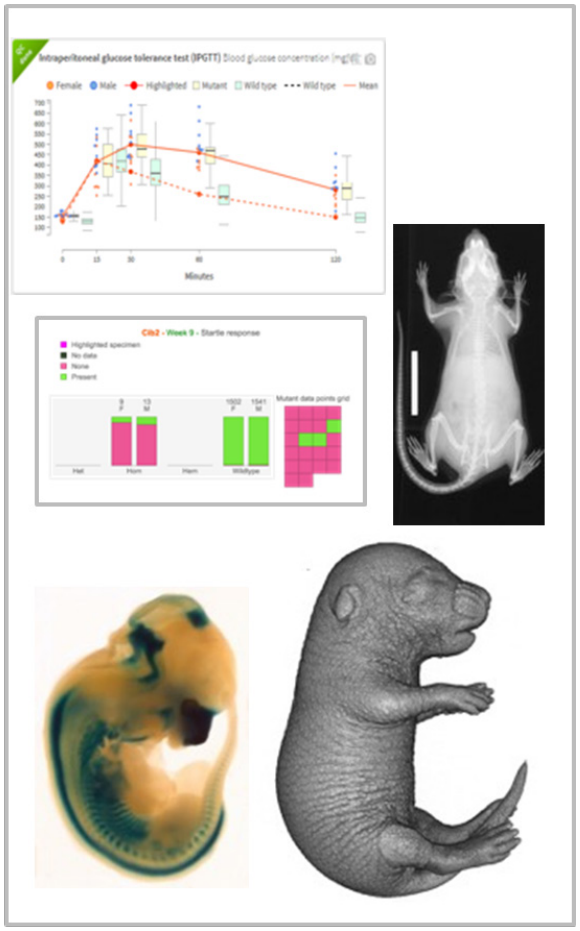
NIH Council of Councils
September 5, 2014
Bldg 31, NIH Campus, Bethesda, MD

The CF KOMP² Project…

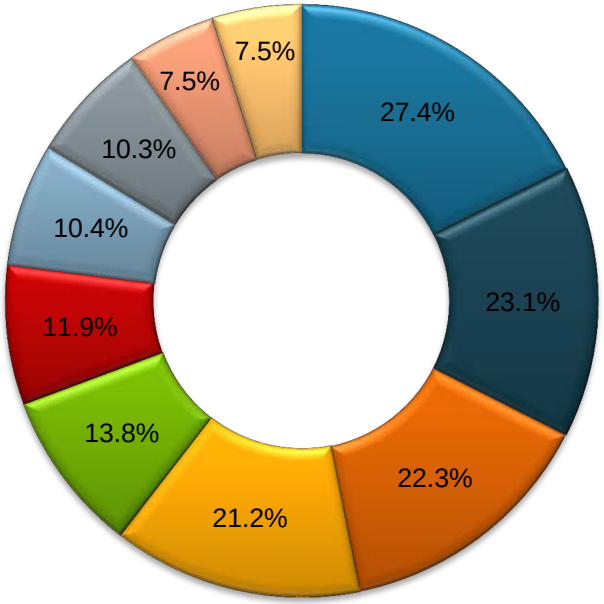
discovering new knowledge about gene function

Today…2017

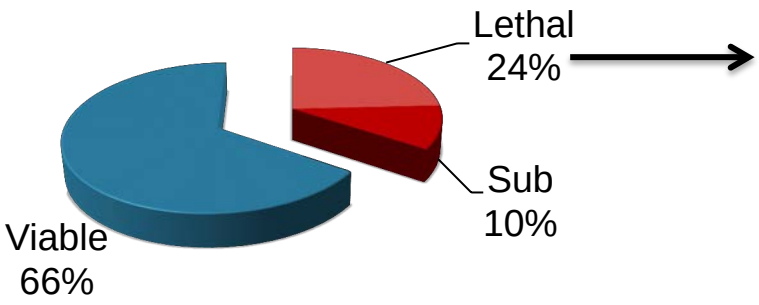
- >5,000 (2000) lines produced (ESC and CRISPR)
- 4,708 (100) lines phenotyped (~144K mice)
- ~500 phenotyping parameters
- >37.5million data points
- 28,406 phenotype annotations
- 270,800 images
- 3,642 (1,200) orders received
- Fully adopted CRISPR/Cas9 exdel allele
- Added late onset phenotyping pipeline
- 34 consortium publications; 1,189 user publications
- Mature organization: 16 centers, 13 countries, 4 continents

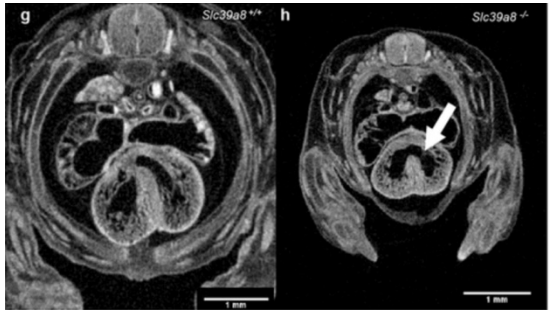


Top 10 adult abnormal phenotypes



About ***one-third*** of IMPC KO strains are ***embryonic lethal or subviable.*** imaging technologies are employed to analyse structural dysmorphologies.





Coronal sections through micro-CT volumes of mutant and control Slc39a8 E14.5 embryos revealed heart morphological defects including ventricular septal defects (white arrow),

M Dickinson *et al.* 2016, *Nature*

KOMP2 Phase 1 Goals

2011- 2015

- Produce 2,500 knockout strains
  - Ensure equal availability to research scientists
- Phenotype 2,500 knockout strains
  - Standardized procedures, harmonized protocols
  - Sex-balanced cohorts, blinded and random testing
  - Lethal knockouts analyzed at embryonic stages
- Disseminate data through Web portal
  - Real-time data access to all, without restriction
  - Include metadata, statistical analysis

KOMP2 Phase 2 Goals

2016- 2021

All Phase 1 goals, plus…

- Produce 3,000* more knockout lines
  - Generated using CRISPR/Cas9 technology
- Phenotype 3,000 more knockout lines
  - 15% of lines analyzed while aging
- Disseminate data through Web portal
  - Provide clinical interpretation and insights

**Original plan: 6,000 more lines*

Reproducibility: Standardized, harmonized protocols

Procedures linked to behaviour/neurological phenotypes
Embryo pipeline
Gross Morphology Embryo E9.5-E15.5
Histopathology Embryo E9.5
Adult pipeline
Acoustic Startle and Pre-pulse Inhibition
Auditory Brain Stem Response
Gross Pathology and Tissue Collection
Brain Histopathology
Organ Weight
Eye Morphology
Indirect ophthalmoscopy
Calorimetry
Electroconvulsive Threshold Testing
Food efficiency
Combined SHIRPA and Dysmorphology
Grip Strength
Hole-board Exploration
Hot Plate
Light-Dark Test
Open Field
Rotarod
Sleep Wake
Slit Lamp
Tail Flick
Tail Suspension

Grip Strength IMPC_GRS_001

- Purpose
- Experimental Design
- Equipment
- Procedure
- Notes
- Parameters
- Metadata

Purpose

The grip strength test is used to measure the neuromuscular function as maximal muscle strength of forelimbs and combined forelimbs and hind limbs. These are assessed by the grasping applied by the mouse on a grid that is connected to a sensor. Three trials are carried out in succession measuring forelimb-strength only, followed by three successive trials measuring the combined forelimb/hindlimb grip strength. All grip strength values obtained are normalized against mouse body weight.

Ontological description: MP:0001515 - abnormal grip strength.

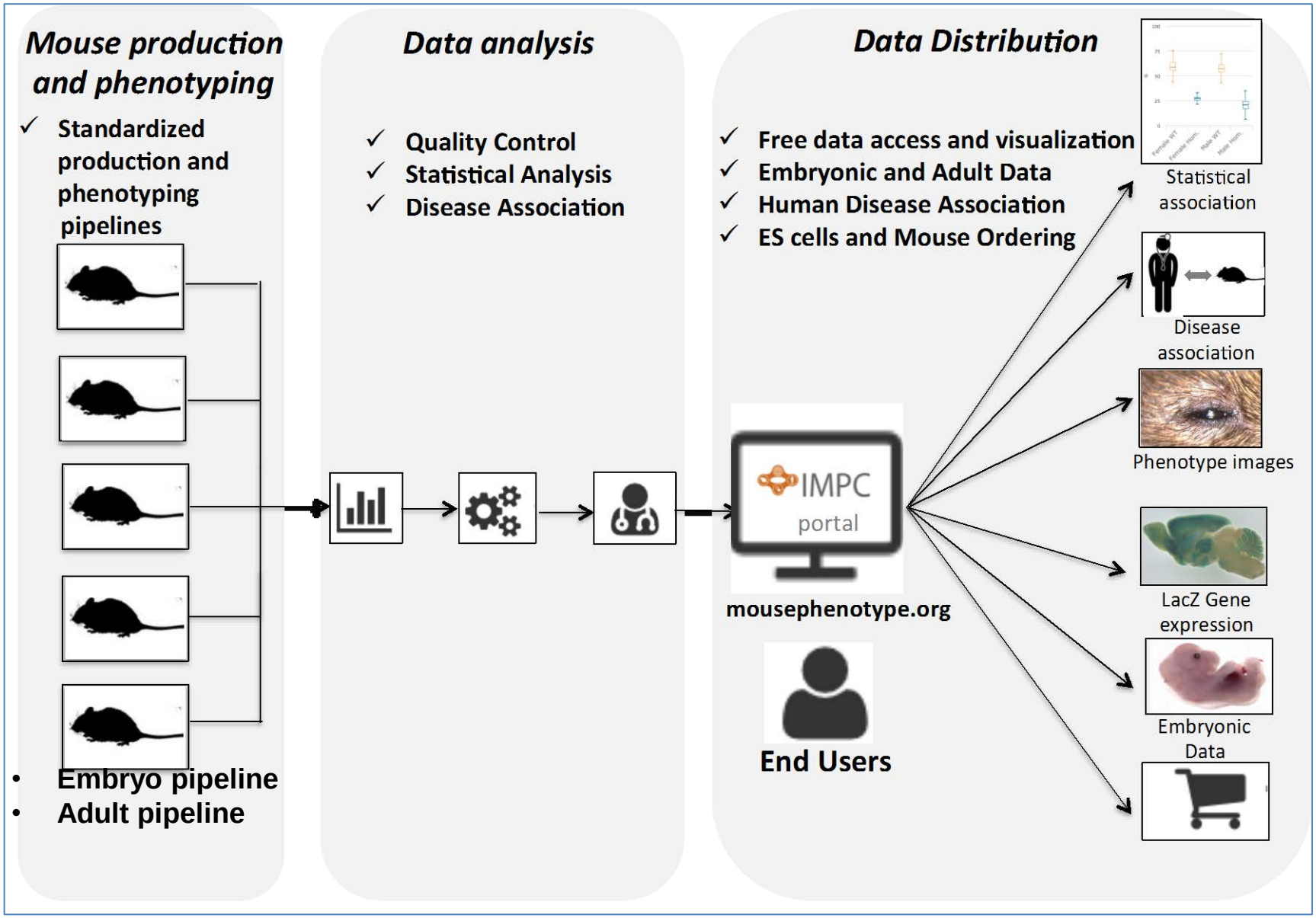
Experimental Design

Minimum number of mutant animals: 7 mice for each sex.

Age of animal: 9 weeks.

Sexual dimorphism: Yes.

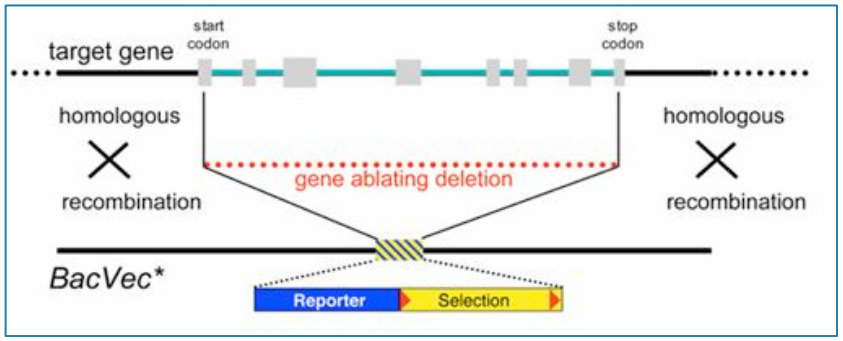
Data flow


Mouse production and phenotyping
✓ Standardized production and phenotyping pipelines
• Embryo pipeline
• Adult pipeline
Data analysis
✓ Quality Control
✓ Statistical Analysis
✓ Disease Association
Data Distribution
✓ Free data access and visualization
✓ Embryonic and Adult Data
✓ Human Disease Association
✓ ES cells and Mouse Ordering
IMPC portal
mousephenotype.org
End Users
Statistical association
Disease association
Phenotype images
LacZ Gene expression
Embryonic Data

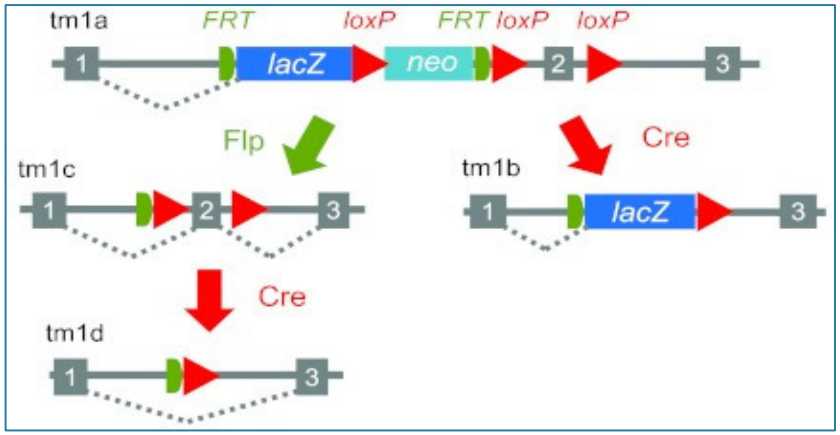
KOMP2 Production:

Phase1

Definitive-null:



Knockout-first:



Phase 1 mice made from alleles generated by homologous recombination in ES cells

Phase2

ExDel:

Intra-exon deletions
- *single-exon genes*
- *single-frame genes*
- *overlapping genes*

4G Whole-exon deletions

- Straight-forward to design
- Efficient
- Deletion of critical exons to generate null
- Screening by end-point PCR
- Screening protocols become genotyping protocols
- QC *via* direct sequencing of deletion amplicon
- Alleles are standardized by design

Phase 2 mice made from newly made exdel alleles generated by CRISPR/Ca9 in zygotes

KOMP2 Production using CRISPR*

*International Microinjection Tracking System

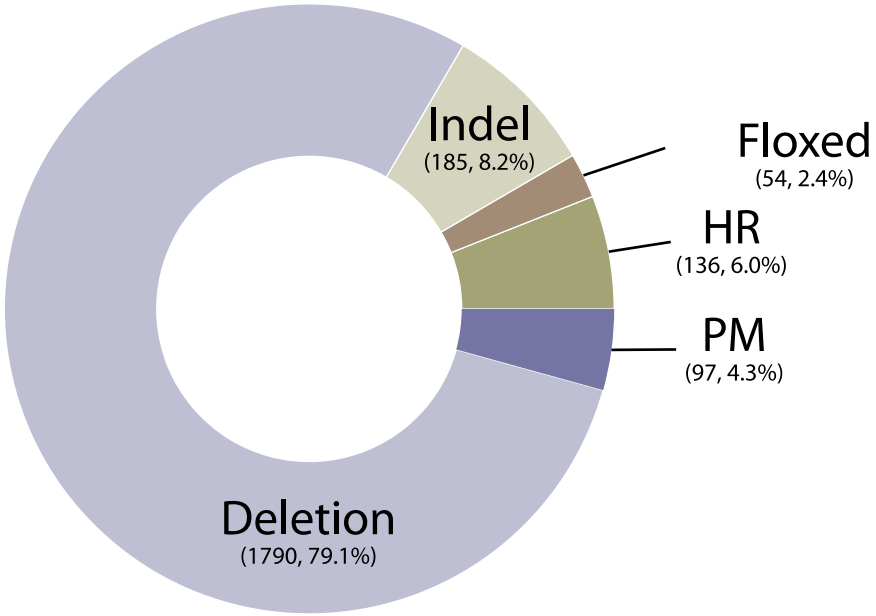
Gene Status Summary – Oct 6, 2016

Status in IMITS*	Unique Genes
Genes assigned	1971
Genes injected	1025
Genes @ microinjection	138
Genes @ founders	120
Genes @ GLT	617
Genes @ MI aborted	152

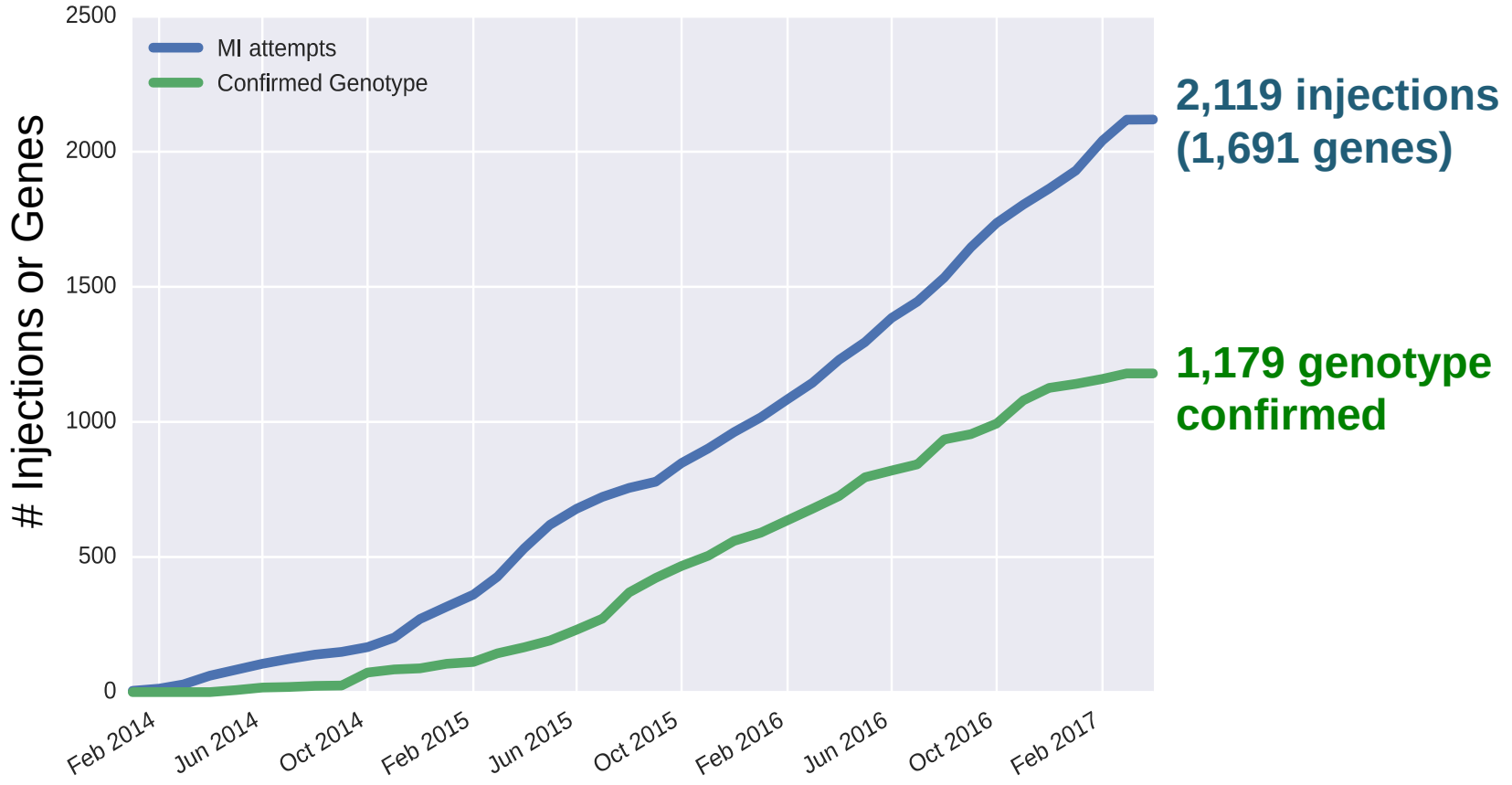
~1.20 injections/gene

80% GLT for genes complete

cf. ~50% GLT for ES cells



KOMP2/IMPC Production by Month

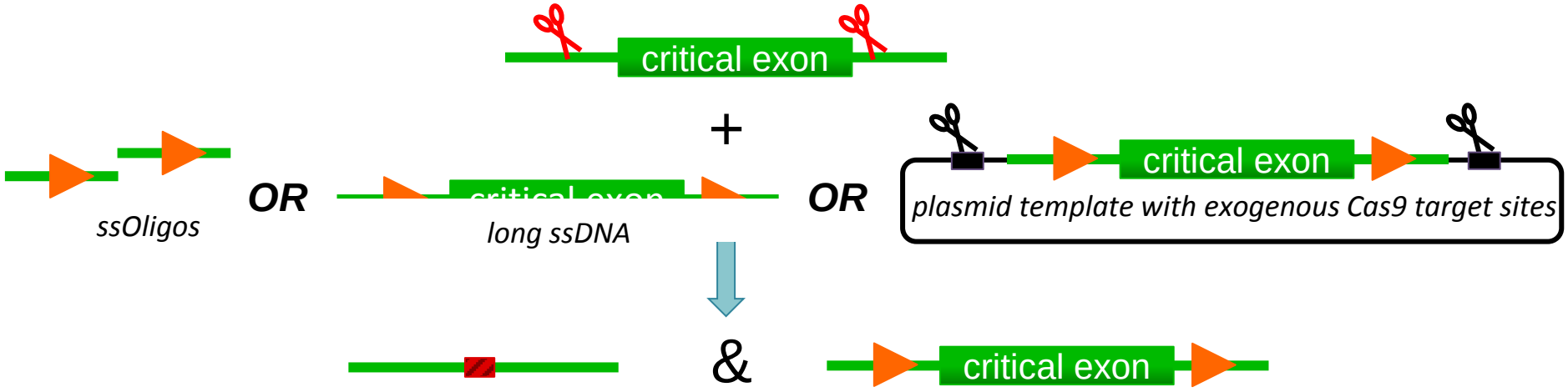


CRISPR adopted, piloted, implemented, high throughput--<2 years

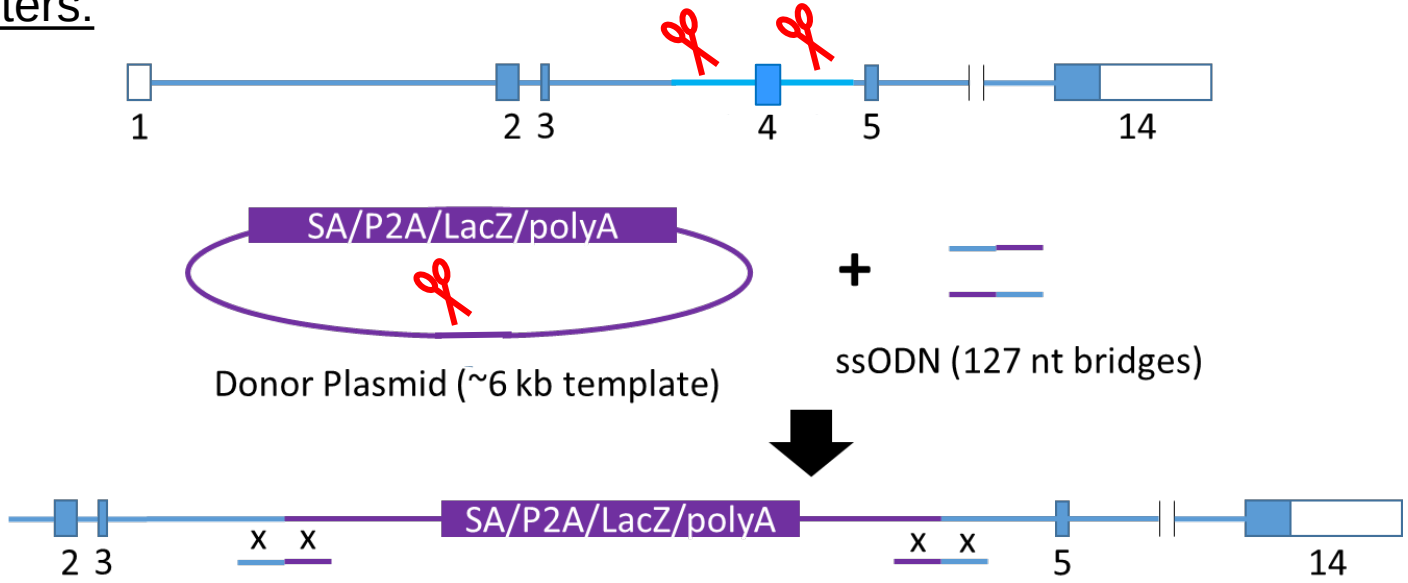
L Nutter, G Clark, 2017, *paper in progress*

Allele Technology Development

Conditionals:



Reporters:



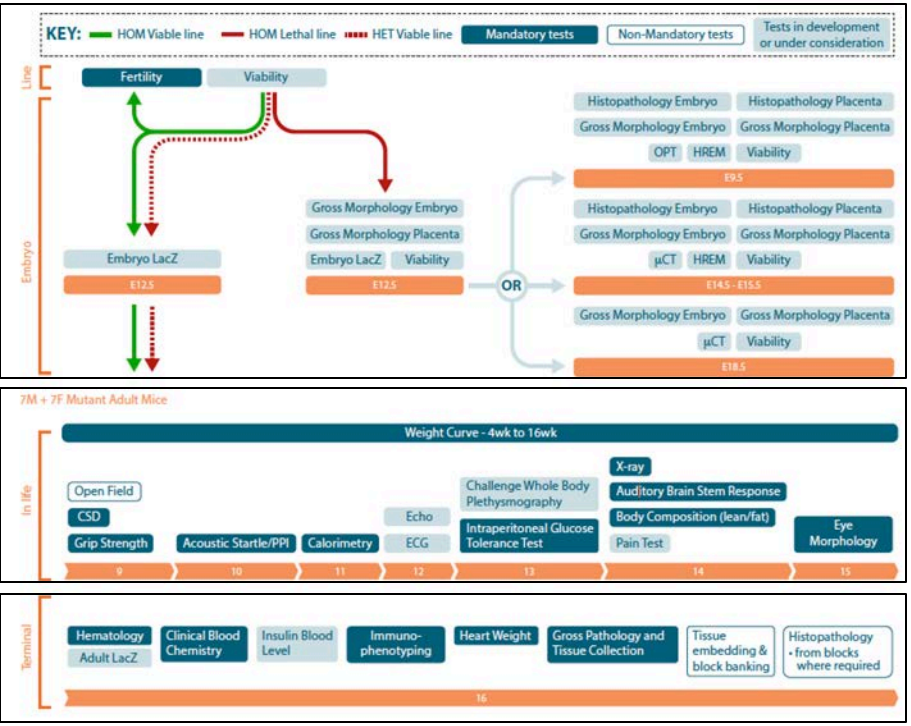
Electroporation

UCD (6 pulses, 3msec, 4G)

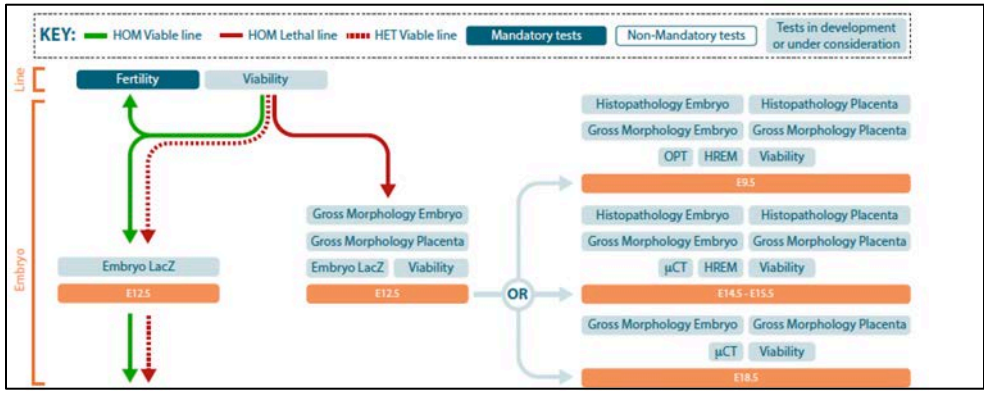
	Genes	Process Efficiency ExDel/ Treated Zyg	ExDel/ Zyg Trns	Process Success ExDel/ Pup
sgRNA/Cas9mRNA/Inj	226	1.7% 229/13231	2.4% of 9603	11% of 2008
sgRNA 8uM/Cas9Prot 8uM/Elec	63	3.6% 118/3274	5.6% of 2113	17% of 714
MR sgRNA 8uM/Cas9Prot 8uM/Elec	14	2.5% 8/320	3.2% of 252	12% of 69
sgRNA 16uM/Cas9Prot 16uM/Elec	**10**	**4.9%** 22/453	**6.2%** of 358	**28%** of 79
mpgRNA 16uM/Cas9Prot 16uM/Elec	**23**	**4.9%** 46/940	**8.5%** of 542	**31%** of 187
sgRNA/Cas9Protein/Inj	12	0.8% 5/619	1.1% of 454	3.3% of 152
mpgRNA/Cas9Protein/Inj	12	3.7% 23/628	5.8% of 400	17% of 138

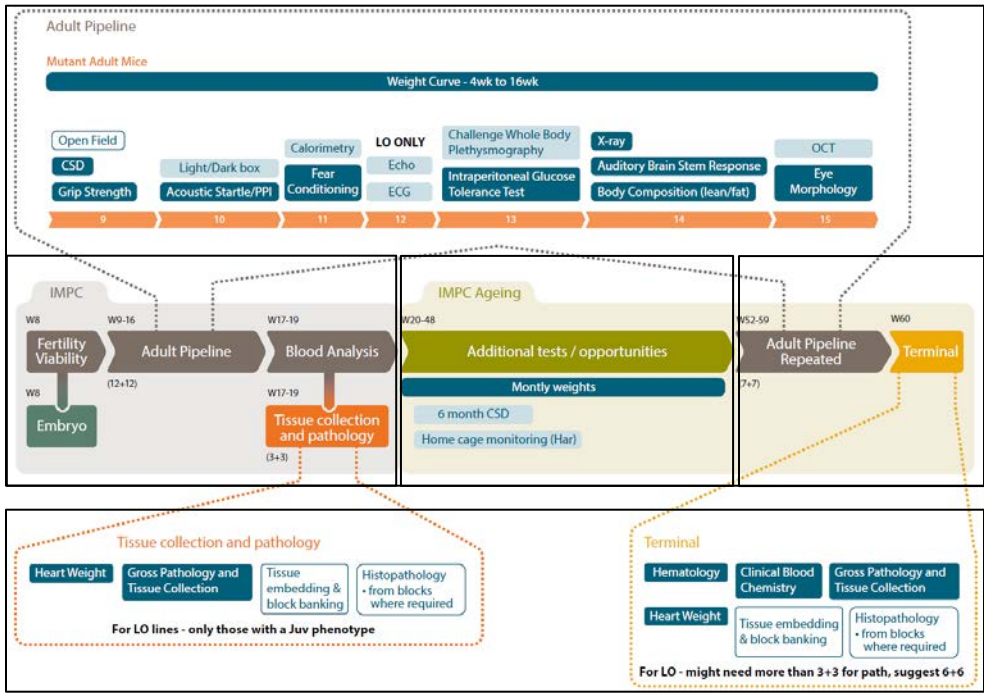
KOMP2 Phenotyping:

Phase1



Phase2

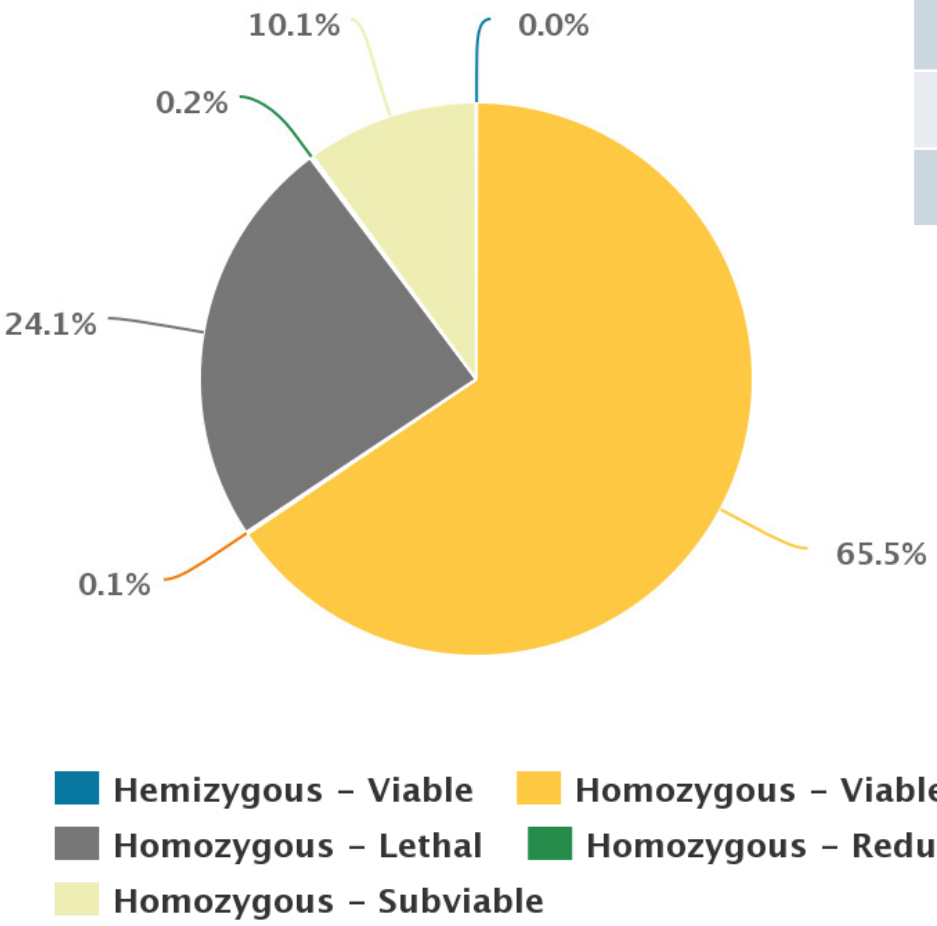




Phase 1 Phenotyping included early adult, embryo, and terminal pipelines

Phase 2 Phenotyping included all Phase 1, plus late adult and intervening pipelines

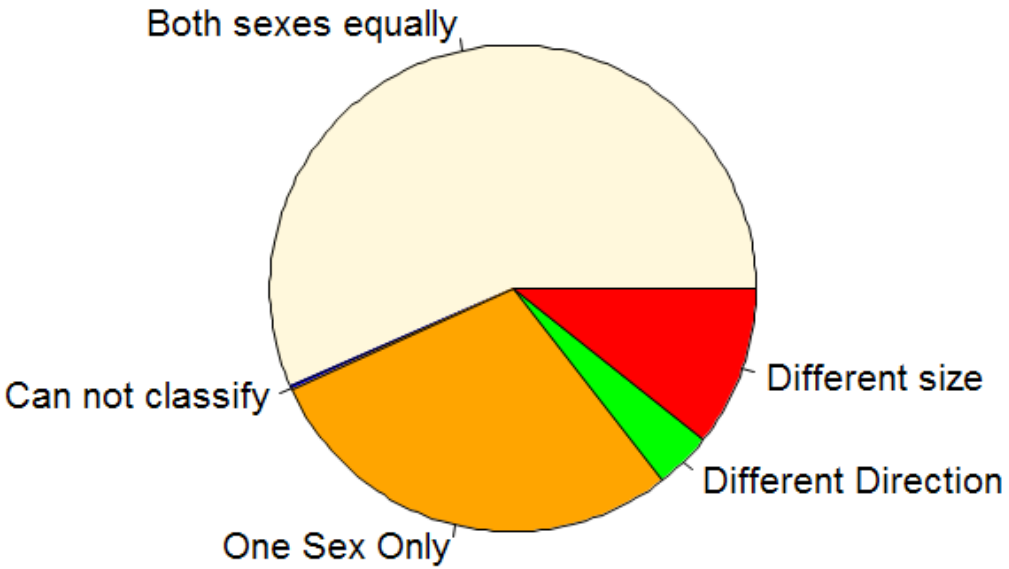
Essential Genes: Revealed



Category	# Genes (% of total)
Viable	1796 (66%)
Lethal	663 (24%)
Subviable	276 (10%)

Sexual Dimorphism: Identified

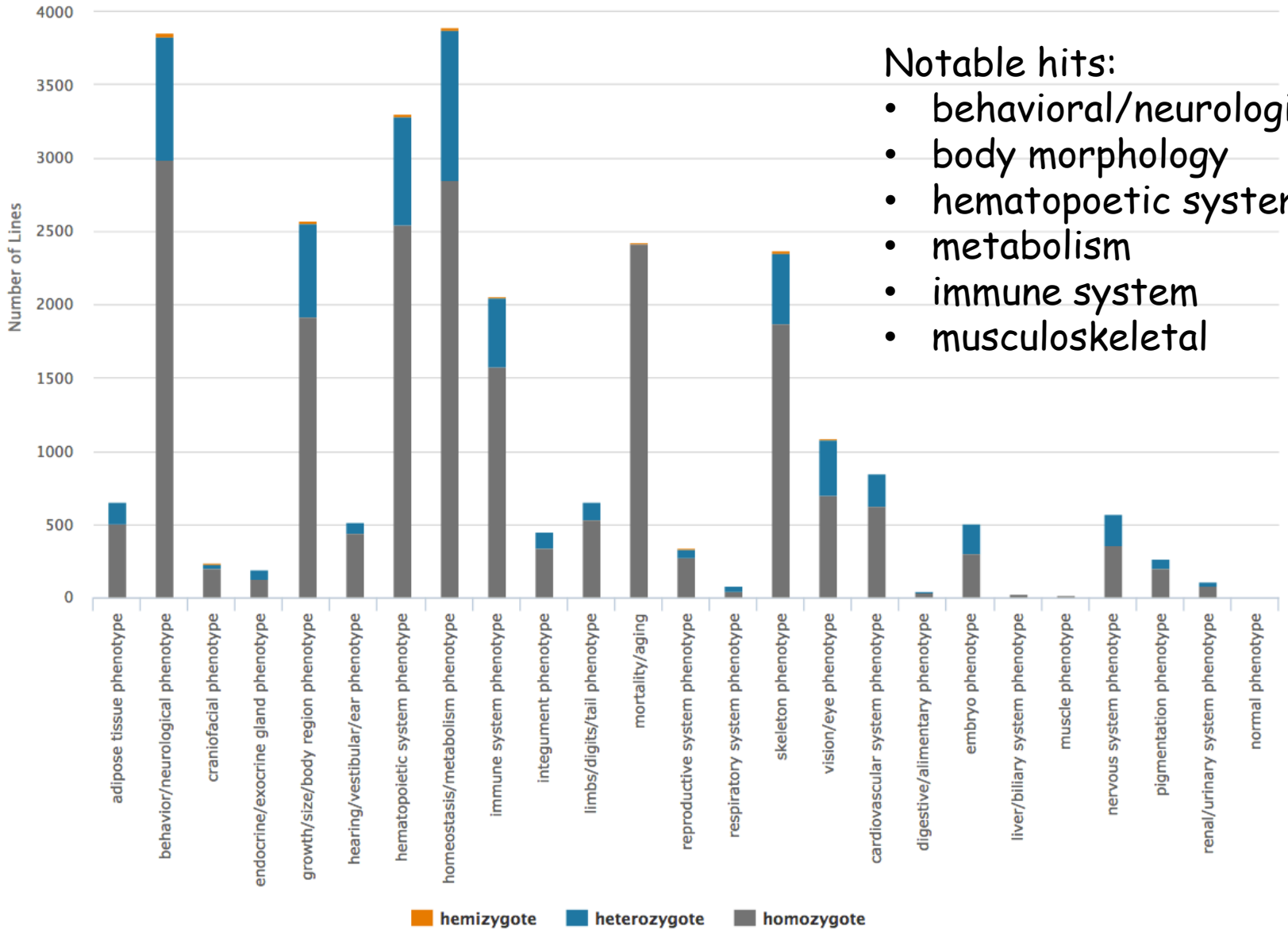
- Phenotypes resulting from a mutant allele in 2.82% of the total tests (87072)
- Almost half display significant sexual dimorphism



Classification	Number	Percent
Both sexes equally	1392	56.6
One sex only	704	28.6
Different size	263	10.7
Different directions	93	3.8
Cannot classify	6	0.2

N Karp *et al.* 2017, *Nature Comms*

Pleiotropy: Discovered

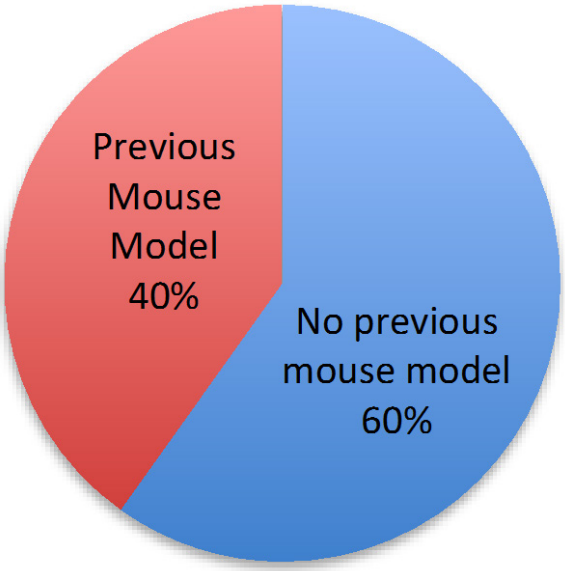


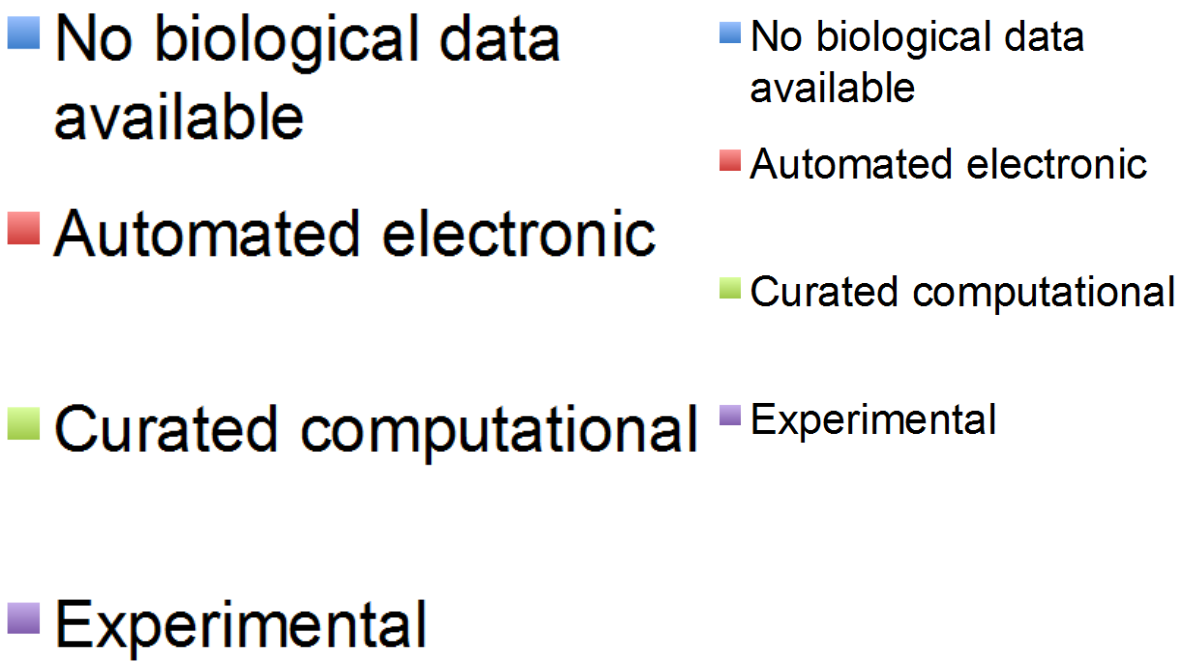
Notable hits:

- behavioral/neurological
- body morphology
- hematopoetic system
- metabolism
- immune system
- musculoskeletal

Prioritizing the unknown

61% of enrolled genes have no available mouse knockout





Of those…47% of genes have no confirmed GO functional annotations

Disease association pipeline

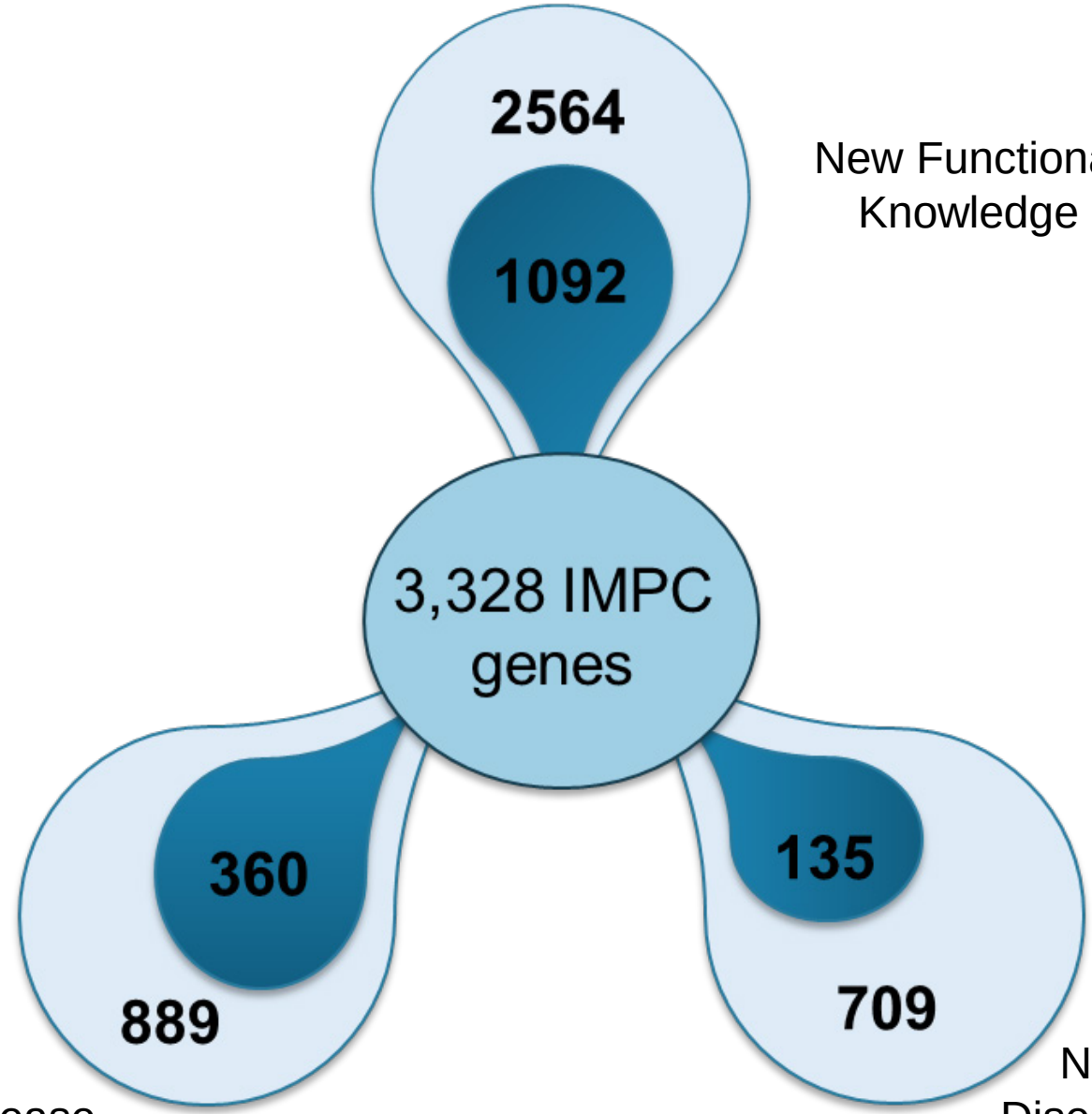
~7000 Mendelian diseases with phenotype annotations (HPO)
- OMIM
- Orphanet
- DECIPHER

Mice with phenotype annotations (MP)
- MGI
- IMPC

Phenotype similarity comparisons PHENO DIGM

IMPC portal
- Mouse models for known disease-gene associations
- Candidates for novel disease-gene associations
- Suggestions for secondary phenotyping projects

Revealing Disease Candidates and New Models



New Functional Knowledge

Models of Mendelian Disease

Novel Mendelian Disease Candidates

D Smedley *et al*, 2017, *Nature*

Disease models across diverse biological systems

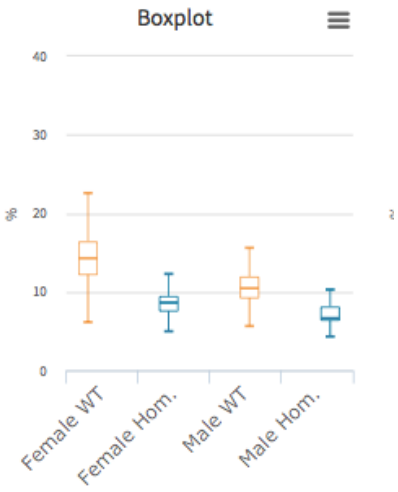
- 185 of 889 (21%) Mendelian disease-gene associations were modelled
- 134 of 185 (72%) **novel** with no previous mouse model reported in literature (MGI)

Biological system	Disease Gene	Human Mendelian disease	Relevant Human Phenotype	Overlapping Mouse phenotype
Bone	*SCARF2*	Van Den Ende-Gupta Syndrome	Long metacarpals	Increased length of long bones
Cardiovascular	*LMNA*	Cardiomyopathy Dilated 1a	Dilated cardiomyopathy	Increased heart weight
Craniofacial	*MSX1*	Orofacial Cleft 5	Cleft palate	Cleft palate
Embryo	*PSPH*	Phosphoserine Phosphatase Deficiency	Intrauterine growth retardation	Abnormal embryo size
Growth/Body size	*GHRHR*	Isolated Growth Hormone Deficiency, Type Ib	Short stature	Decreased body length
Hearing	*SLC52A2*	Brown-Vialetto-Van Laere Syndrome 2	Sensorineural hearing impairment	Increased or absent threshold for auditory brainstem response
Hematopoietic	*GP9*	Bernard-Soulier Syndrome	Thrombocytopenia	Thrombocytopenia
Metabolism	*KCNJ11*	Diabetes Mellitus, Noninsulin-Dependent	Type II diabetes mellitus	Impaired glucose tolerance
Muscle	*COL6A2*	Bethlem Myopathy	Distal muscle weakness	Decreased grip strength
Neurological	*GOSR2*	Epilepsy, Progressive Myoclonic, 6	Difficulty walking	Abnormal gait
Reproductive System	*RNF216*	Gordon Holmes Syndrome	Infertility	Male infertility
Retina	*BBS5*	Bardet-Biedl Syndrome 5	Rod-cone dystrophy	Abnormal retina morphology

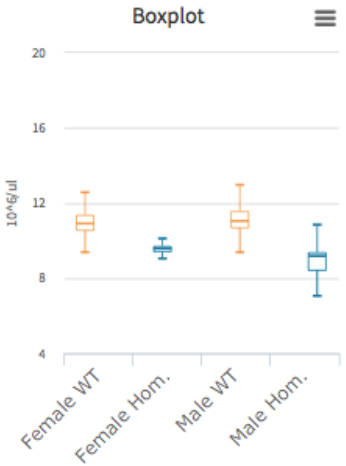
Fbx07: *New phenotype*

- *MGI GO* biological process: negative regulation of lymphocyte differentiation
- KOMP phenotype: *CBC*, clinical blood chemistry, male infertility

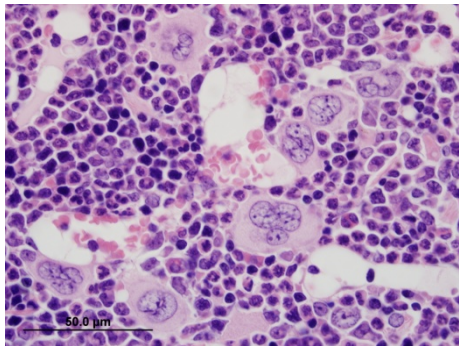
RBCs



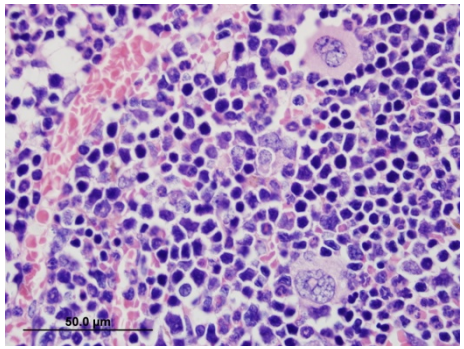
CD3+ T cells



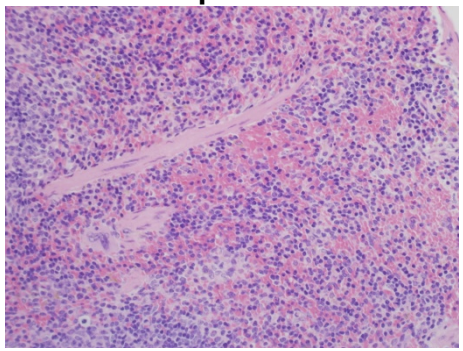
WT bone marrow 100x



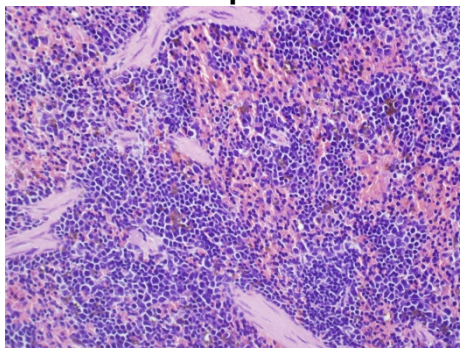
***Fbxo7*$^{-/-}$ bone marrow 100x**



WT spleen 40x



***Fbxo7*$^{-/-}$ spleen 40x**



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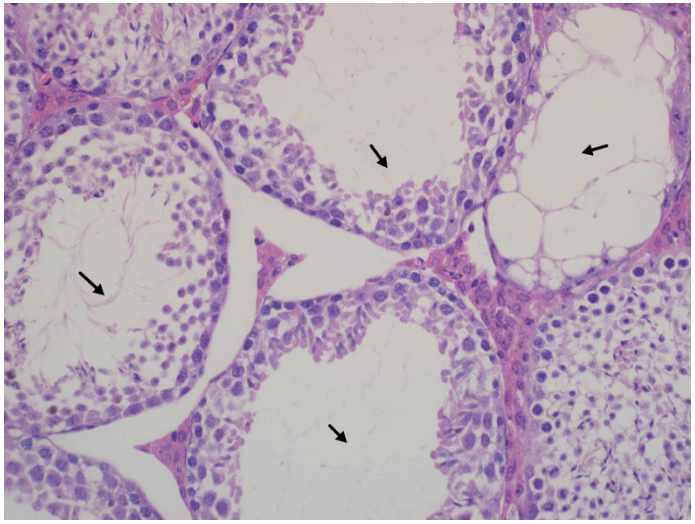
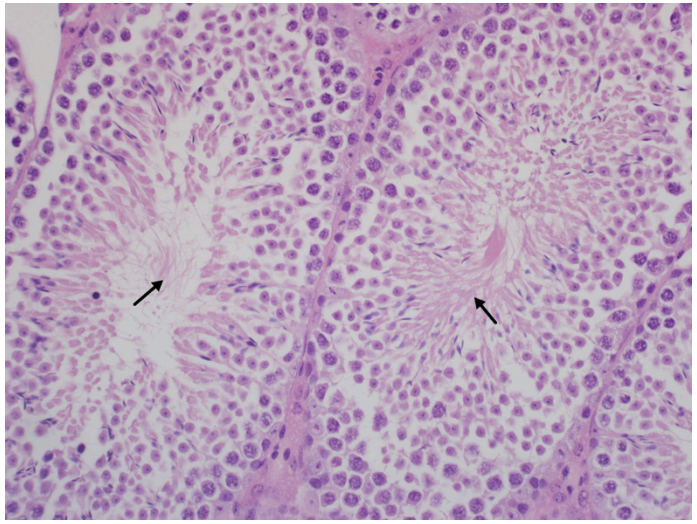
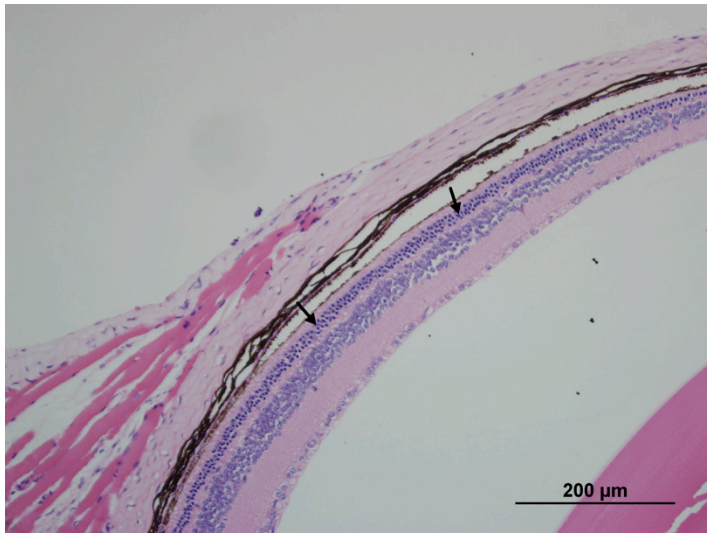


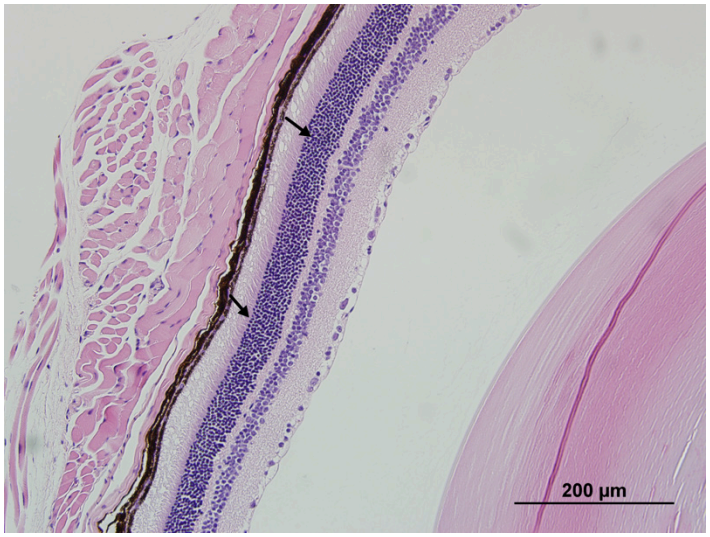


+/+

Seminiferous dilation with minimal spermiogenesis; epididymal aspermia

-/-



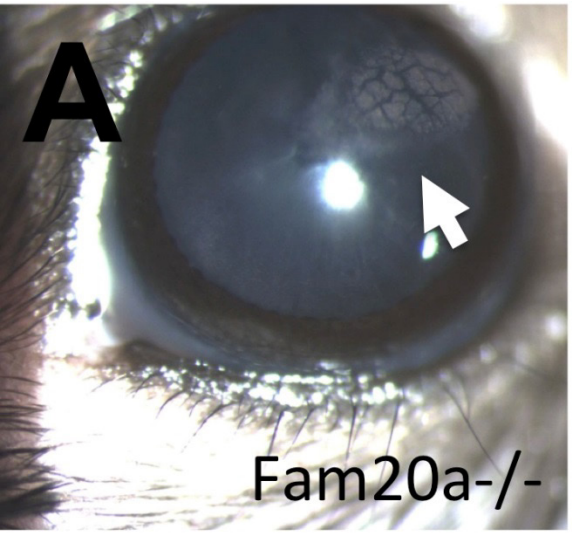


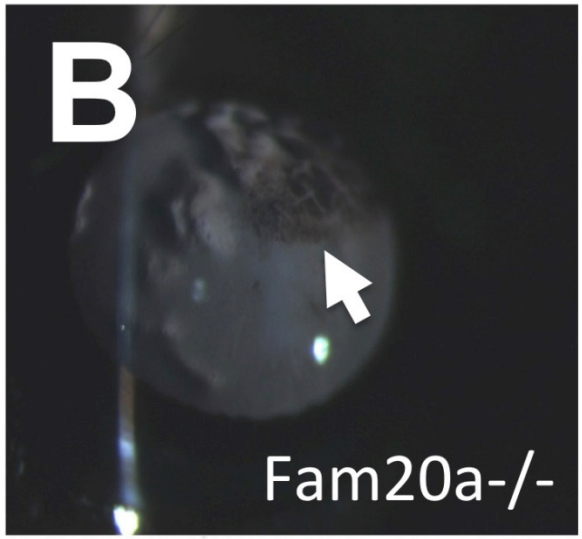
+/+

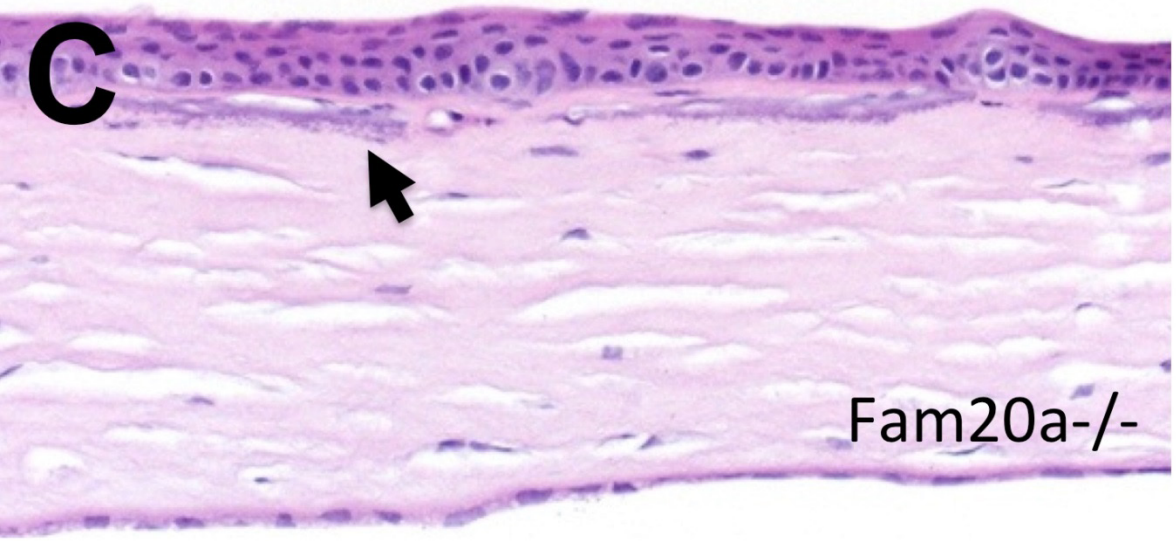
Atrophy of the outer nuclear and outer plexiform layers

Fam20a:
Additional phenotype

Cornea
(NOVEL Gene)







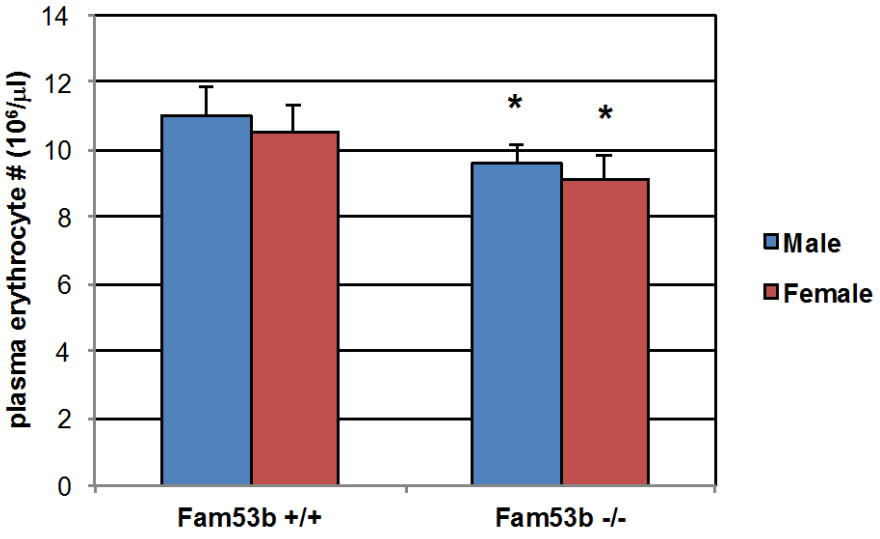
FAM20A (Family with sequence similarity 20, member A)
-MGI:2388266
-OMIM:204690
-DOID:0110066
-Enamel-Renal Syndrome:
-fail to form proper dental enamel ("amelogenesis imperfecta").
-exhibit nephrocalcinosis and nephrolithiasis.
-previous K/O mice show no ocular phenotype

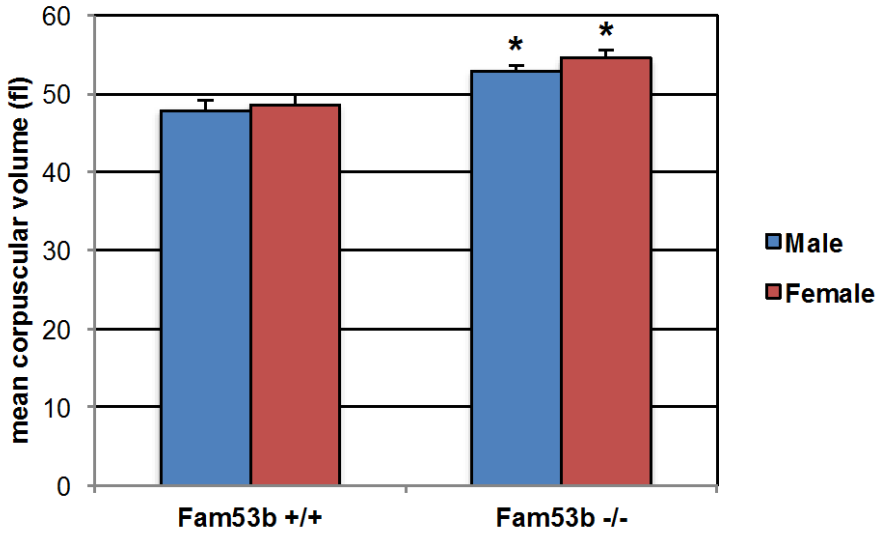
KOMP2 (DTCC): Fam20a$^{-/-}$
(A) Corneal abnormalities in the form of crocodile shagreen evident on biomicroscopy
(B) Retroillumination
(C) Anterior stromal thinning & calcification of the cornea
-males and females
-Abnormal dental morphology

A Moshiri *et al*, 2017, *paper in progress*

Fam53b: *new functional knowledge*

- No reported phenotypes in human or mouse
- Differentially expressed in adult erythrocytes compared to primitive erythrocytes, possible role in Wnt signaling, stem cell maintenance
- Phenotyping suggests role in Diamond-Blackfan Anemia (OMIM:105650) => *possible explanation and alternative pathway for the 46% of cases that are not explained by known mutations in 15 ribosome synthesis genes*



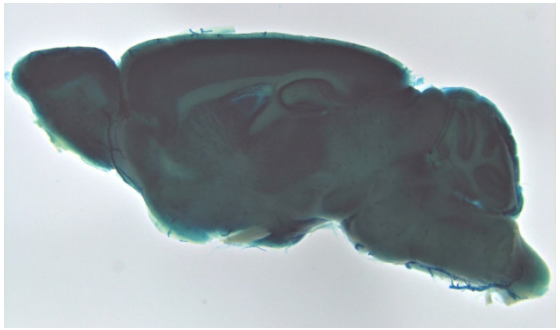


Atp2a2: *pleiotropy*

- Atp2a2 encodes for a sarcoplasmic/endoplasmic reticulum Ca^{++}-ATPase
- Expressed in muscle and brain.
- GWAS between schizophrenia and two SNPs in ATP2A2 (Ripke et al; 2014)
- ATP2a2 associated with Darrier-White disease (OMIM:124200), a dominantly inherited skin disorder that also has a high incidence of psychoses and affective disorders (Jacobsen et al; 1999)

KOMP2:

- *Atp2a2*$^{tm1b(EUCOM)Hmgu}$ homozygous lethal at e12.5
- *Atp2a2*$^{tm1b(EUCOM)Hmgu}$ strongly expressed in heterozygous adult brain
- *Atp2a2*$^{tm1b(EUCOM)Hmgu}$ heterozygous mice present abnormal sensory capabilities/reflexes/nociception and altered prepulse inhibition
- Altered prepulse inhibition is hallmark behavioral phenotype of schizophrenia, which supports association between ATP2A2 linkage findings for schizophrenia / psychoses processes

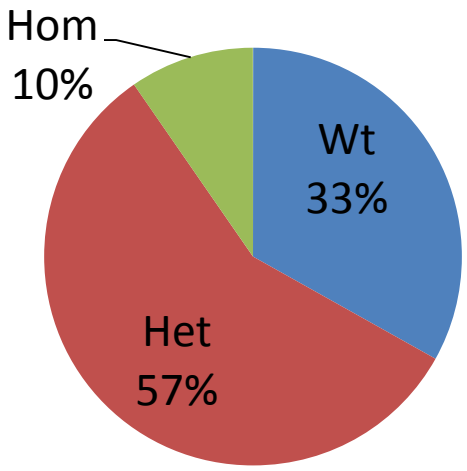


Frrs1l: *pleiotropy & sexual dimorphism*

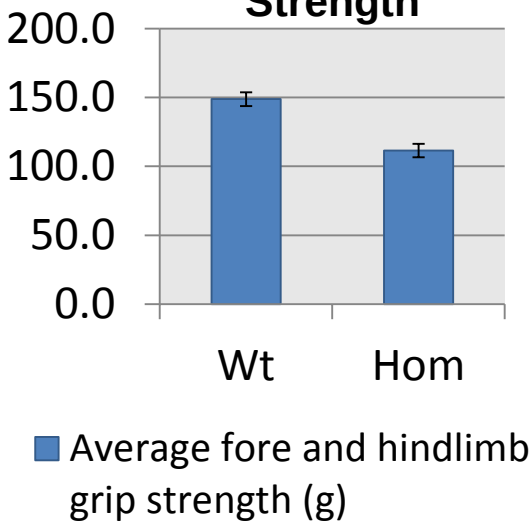
-ferric-chelate reductase 1 like (MGI: 2442704)
-encodes outer-core component of AMPA receptor in brain
-protein thought to interact with inner-core components of receptor
-plays role in modulation of glutamate signaling
-mutations associated with Early Infantile Epileptic Encephalopathy (OMIM: 308350)

KOMP2: ***subviable, extensive pleiotropy, sexual dimorphism***

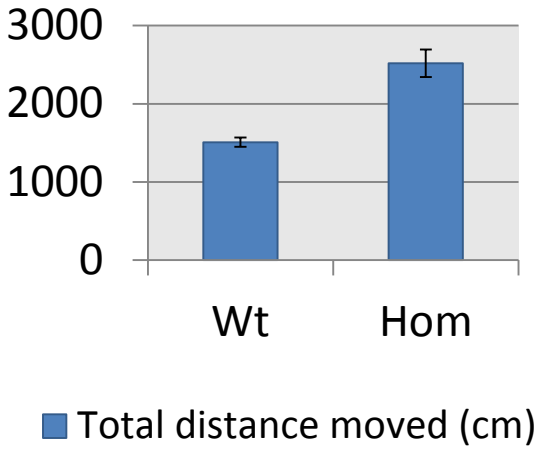
Reduced survival



Reduced Grip Strength



Hyperactivity in open field



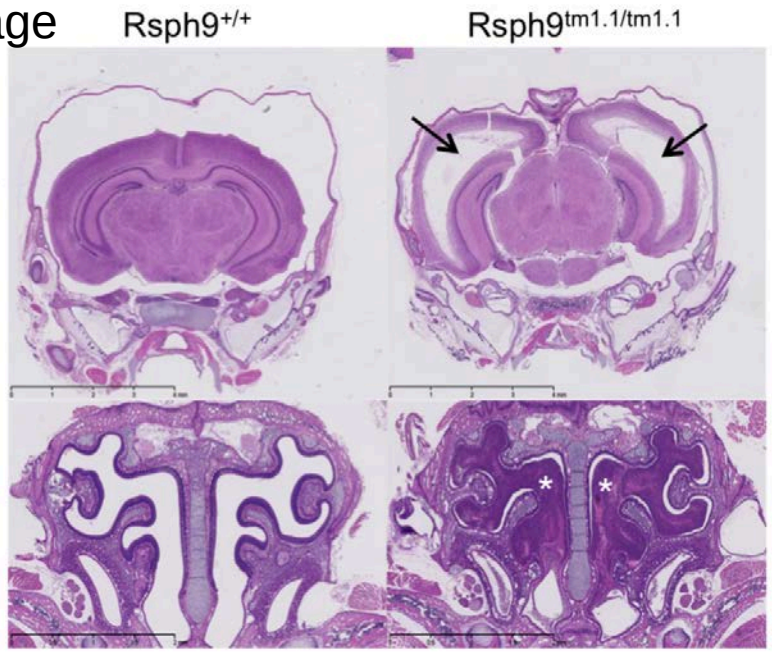
Rsph9: *model of ciliopathy*

- Radial spoke head protein 9 (component of radial spoke head in motile cilia and flagella)
- Functional role in neural and neurosensory cilia (zebrafish studies)
- *RSPH9* mutations identified in patients with Primary Ciliary Dyskinesia

KOMP2:

- IMPC Rsph9 mutants showed partial pre-weaning lethality but viable to P7.
- Whole brain MRI and H&E staining of coronal sections of the P7 brain reveal severe **hydrocephaly** of the left and right lateral ventricles.
- Mice also presented blocked sinuses.

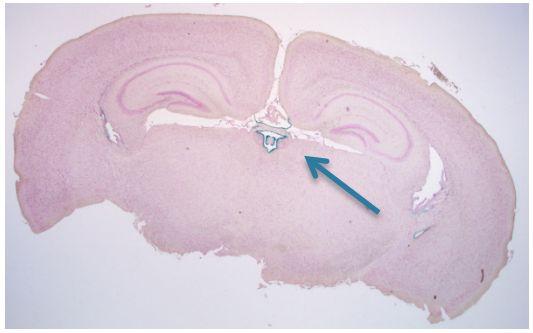
P7 stage



H&E stained coronal sections of P7 mice revealed enlarged ventricles and blocked sinuses in the Rsph9$^{tm1.1/tm1.1}$ mutant mice.

Dickinson et al. 2016, Nature

Adult LacZ expression



Rsph9 HET
Brain expression
(3rd ventricule area

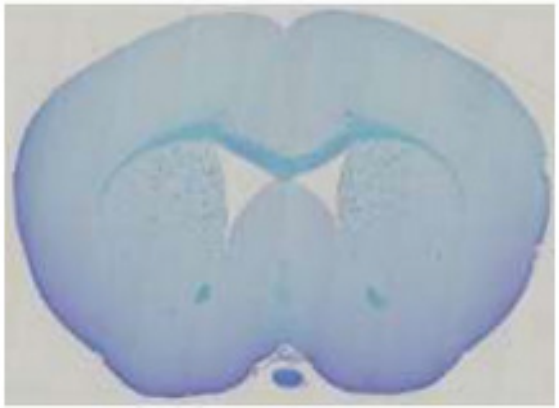
- Rsph9 mouse model recapitulates features of Primary Ciliary Dyskinesia in humans (blocked sinuses and hydrocephaly).
- Expression pattern consistent with phenotype

Aff3: *model of pediatric disease*

- AFF family of putative transcription factors involved in infant acute leukemia and intellectual disability (ID).
- Aff3 required for normal cellular migration in developing cortex (Moore and al; 2014)
- Gene silencing associated with ID and hypotonia at the folate-sensitive fragile site (FSFS) FRA2A (Metsu et al; 2014)

KOMP2:
- Aff3tm1a(EUCOMM)Wtsi are homozygous viable with decreased grip strength

- Brain histopathology: enlarged ventricules, decreased size of corpus callosum



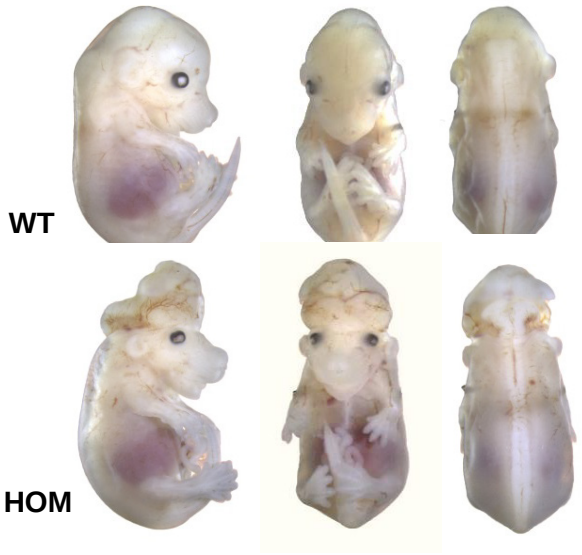
- Adult Aff3 Brain expression

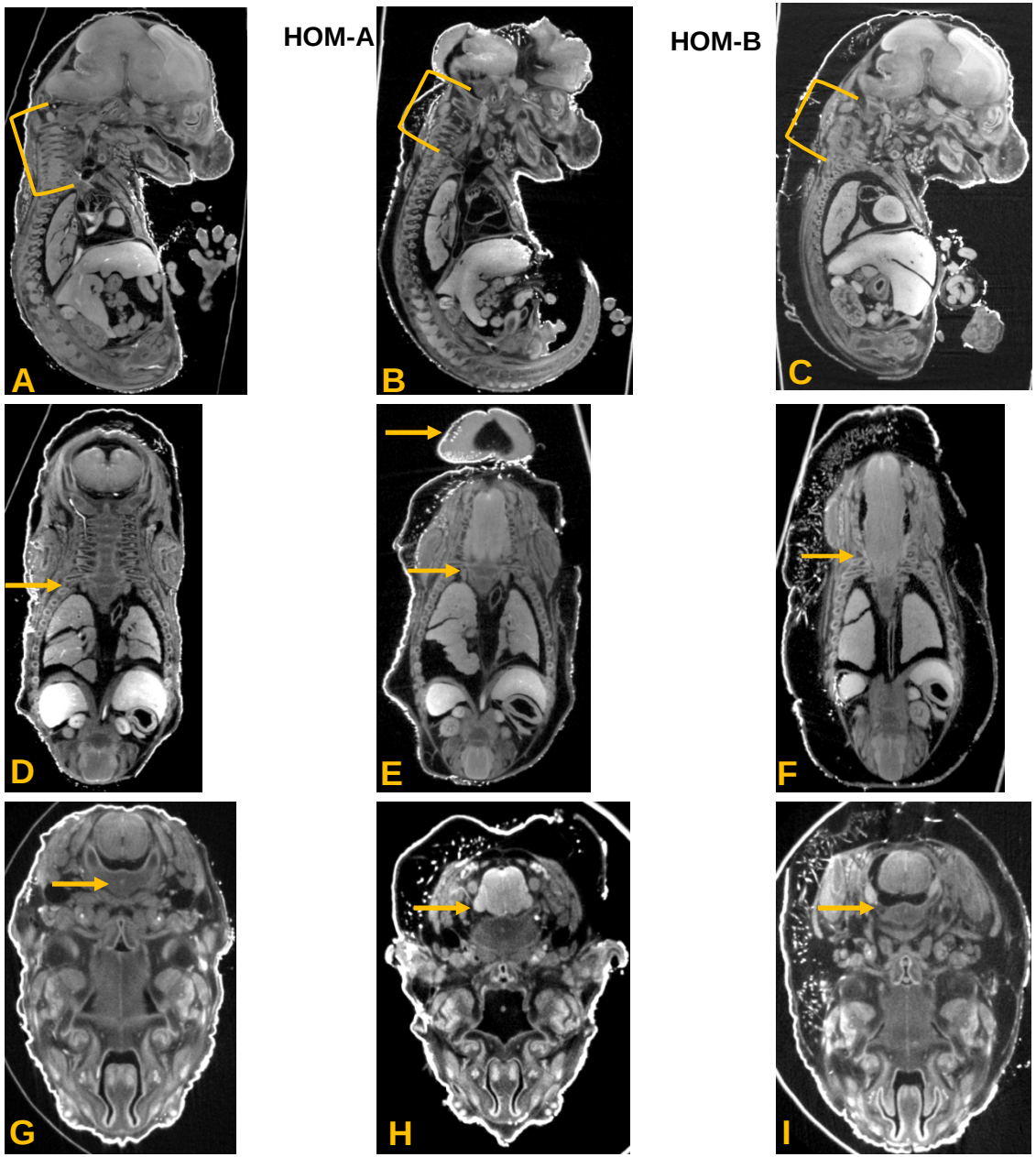
- Corpus callosum abnormalities: common brain malformations, wide clinical spectrum (severe intellectual disability to normal cognitive function)

Aff3 mouse mutant presents features common in FRA2A patients

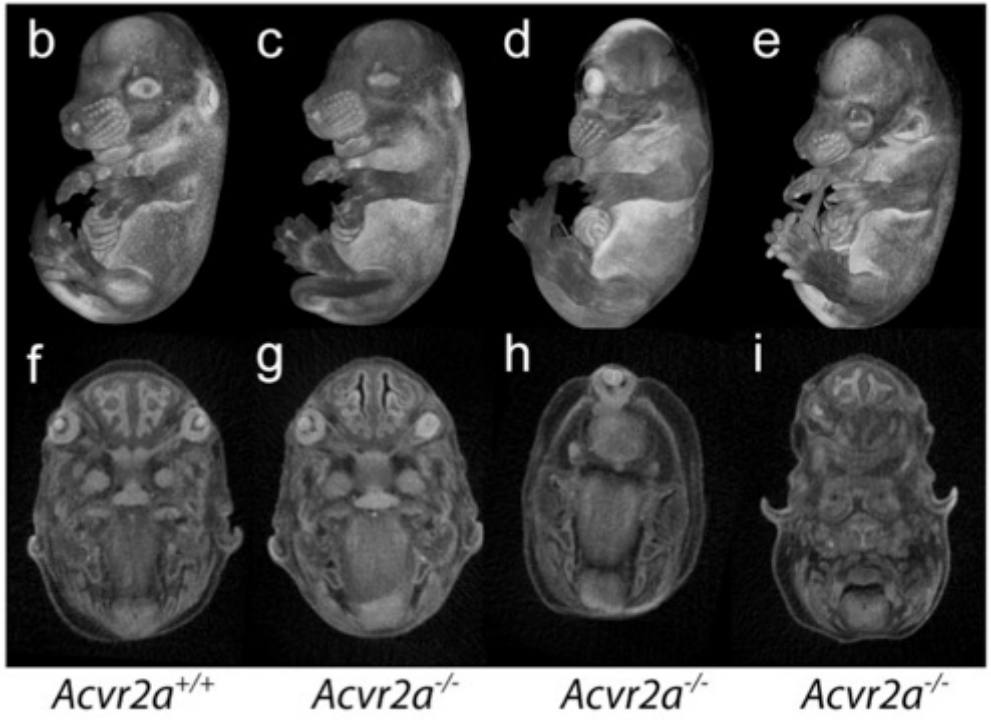
Cnnm2: *Lethal mid-gestation*

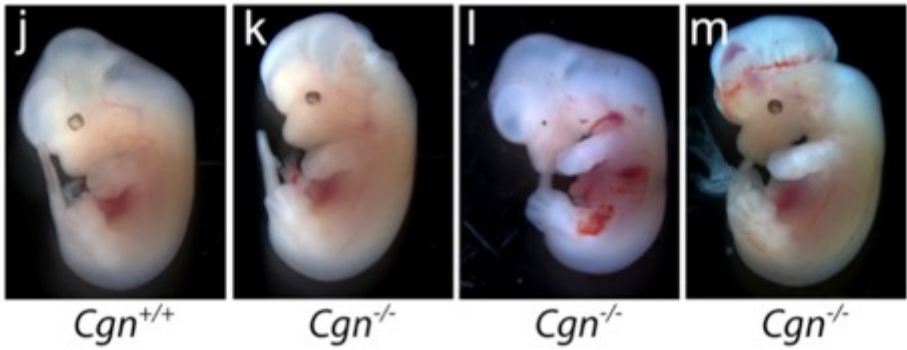
- **Extant knowledge:**
  - Human ortholog: cyclin and CBS domain divalent metal cation transport mediator 2; hypomagnesemia, seizures, mental retardation
- **KOMP:**
  - HOM Lethal at e15.5
  - LacZ e12.5: no stain
  - uCT e15.5: Exencephaly, Heart defects (DORV, VSD, pulmonary trunk, etc), Vertebrae: Cervical C1-C7, hydrops

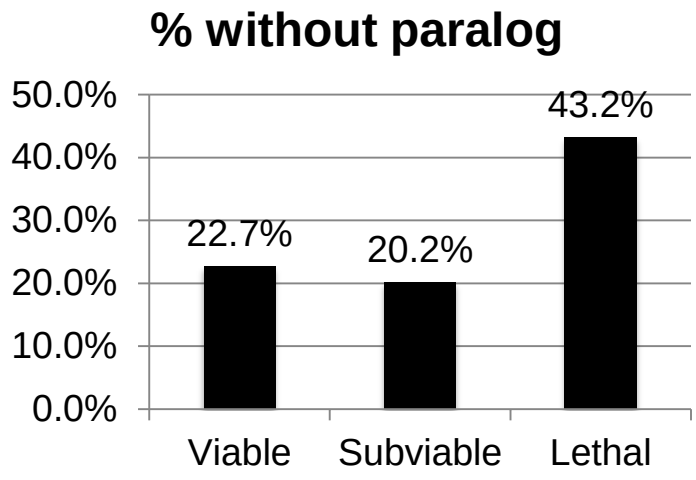




Paralogs: Subviability and incomplete penetrance







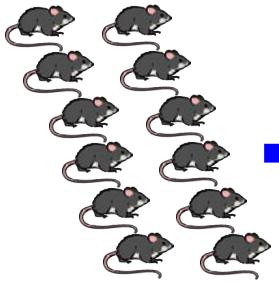
Subviable genes are much less likely to lack a paralog than lethal genes

Challenge Phenotyping: Immune response

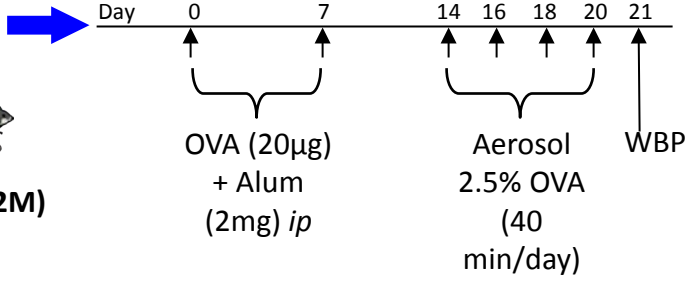
Svs2 - Seminal vesicle secretory protein 2

- GO functional annotation: fertilization, sperm capacitation

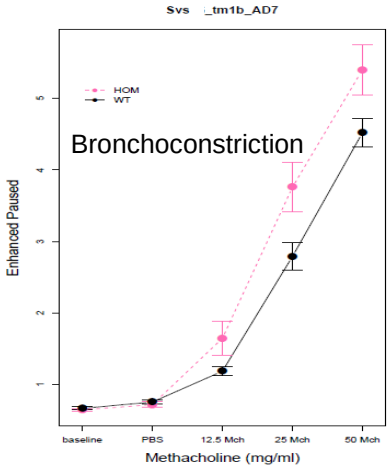
Mutants (6F + 6M)

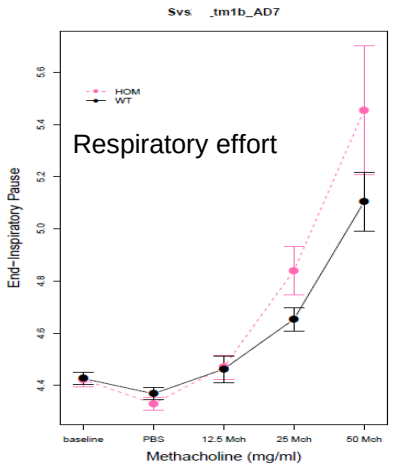


WT Controls (2F + 2M)

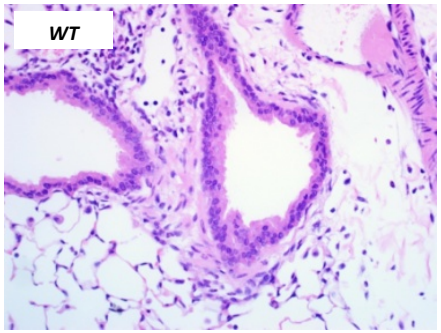


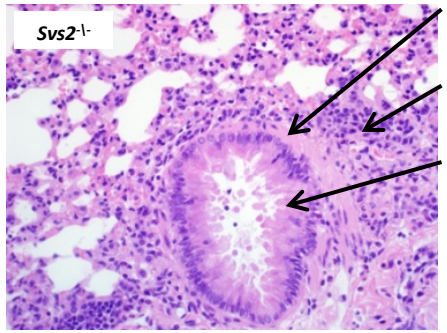
Respiratory Function, Lung Development, and Airway Hyper-reactivity (bronchoconstriction)





Histopathology (allergic airway disease)





Airway wall thickening

Peribronchiolar inflammation

Goblet cell hyperplasia
Mucus secretion

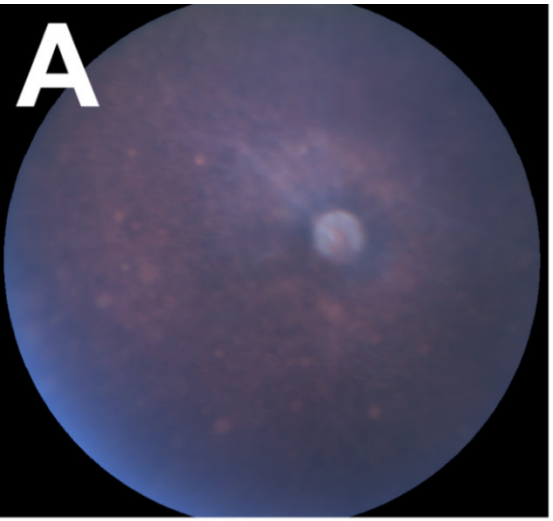
BAL (Cytokines)
Serum Ig (OVA-specific IgE & OVA-specific IgG_1)

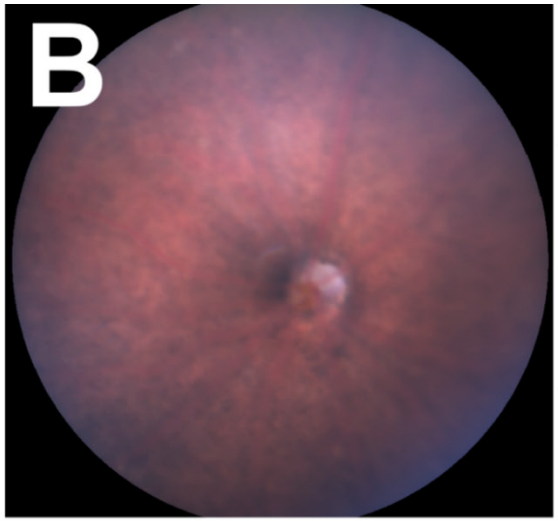
Arap1: *New disease mechanism*

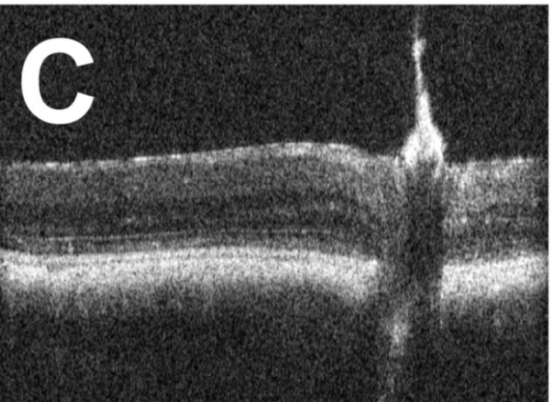
Retina
(VERIFIED Gene)

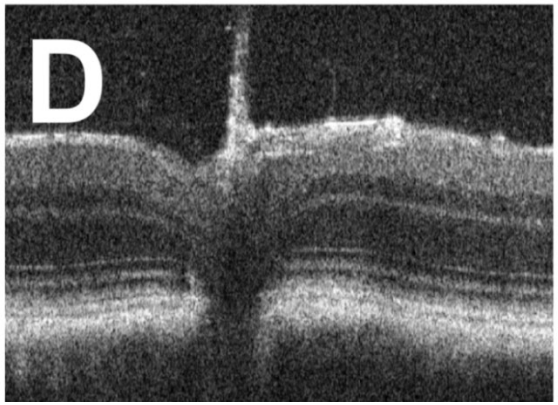
ARAP1 (ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 1)
-MGI:1916960
-OMIM:606646
-regulates lysosome maturation, cell signaling
-extent of functions not well understood.

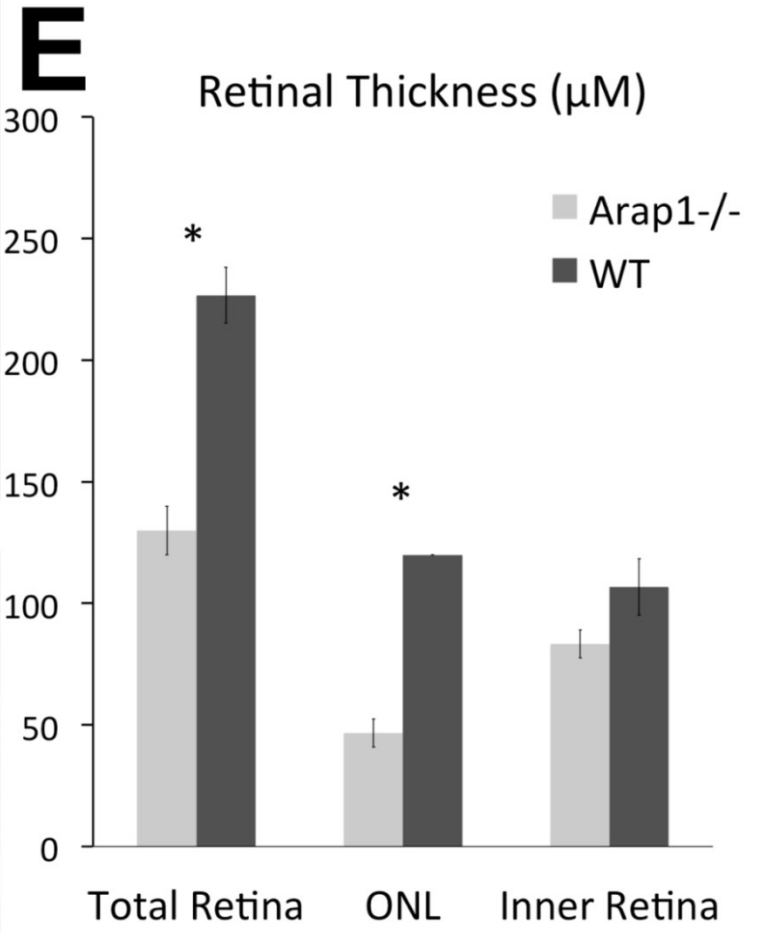
KOMP2 (DTCC): Arap1$^{-/-}$
(A) Mutant fundus (16 wks, male)
(B) Wildtype fundus (16 wks, male)
(C) Mutant OCT (16 wks, male)
(D) Wildtype OCT (16 wks, male)
(E) Retinal thickness
-male and female



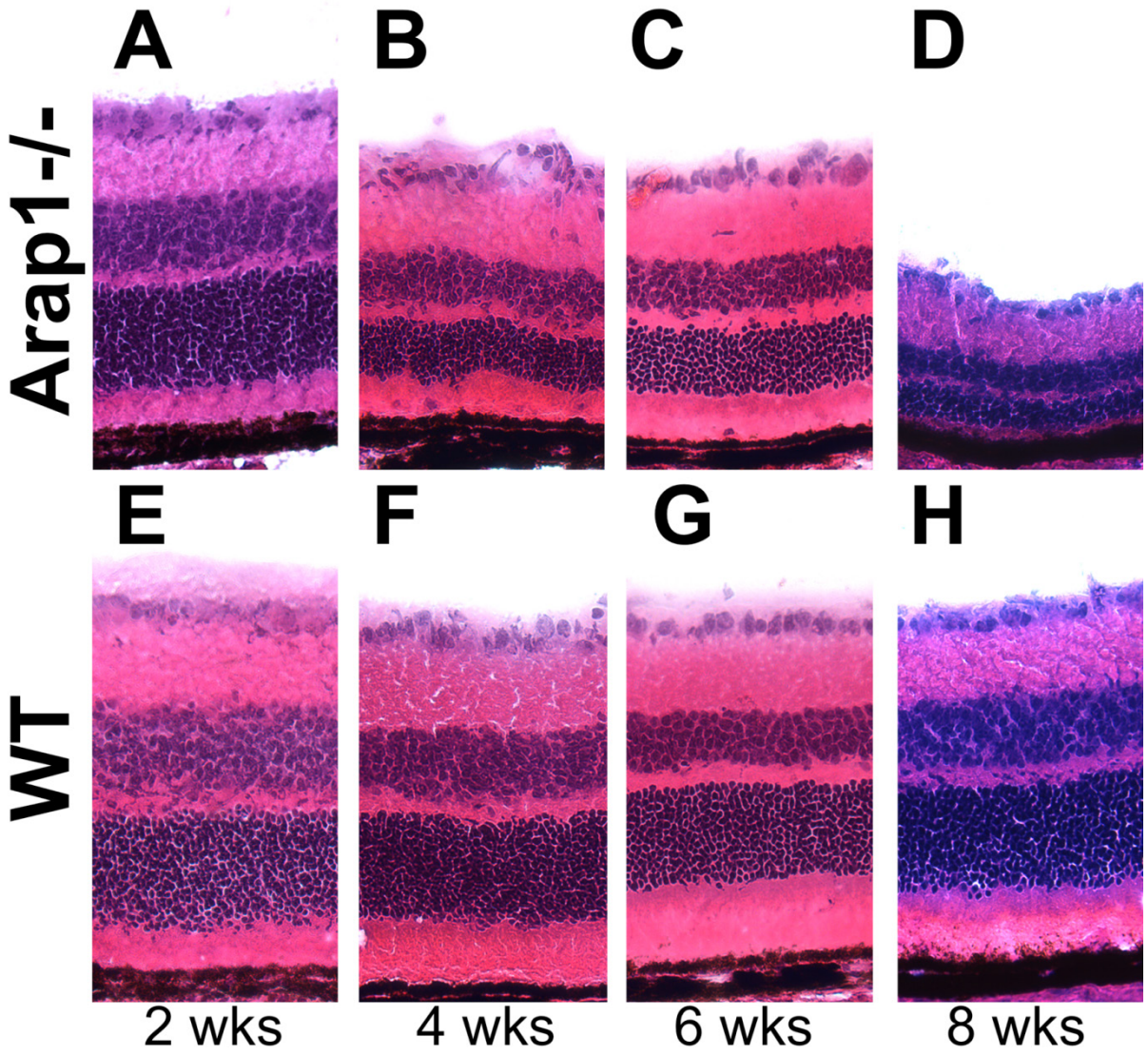






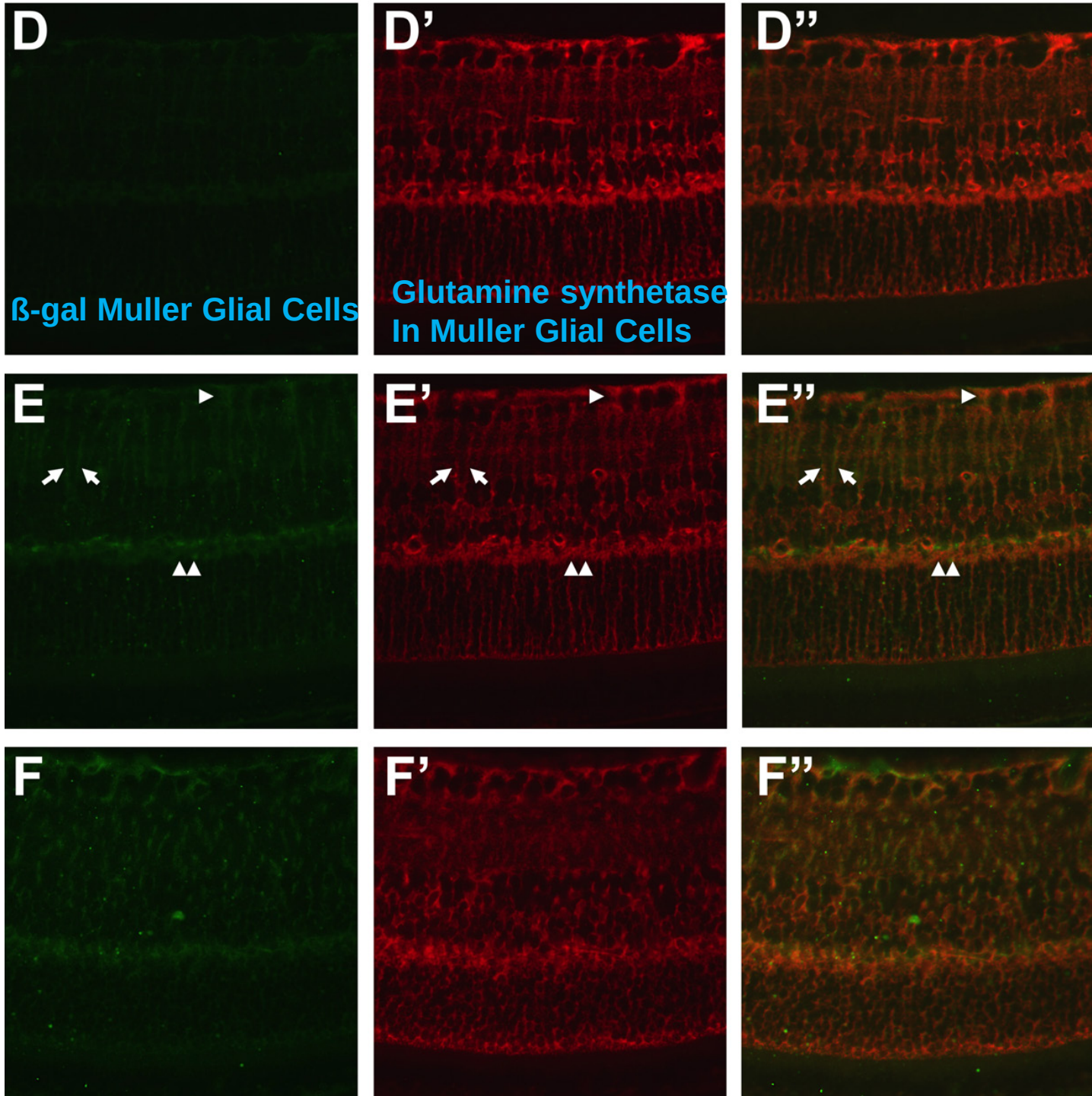


A Moshiri *et al*, 2017, *IVOS*

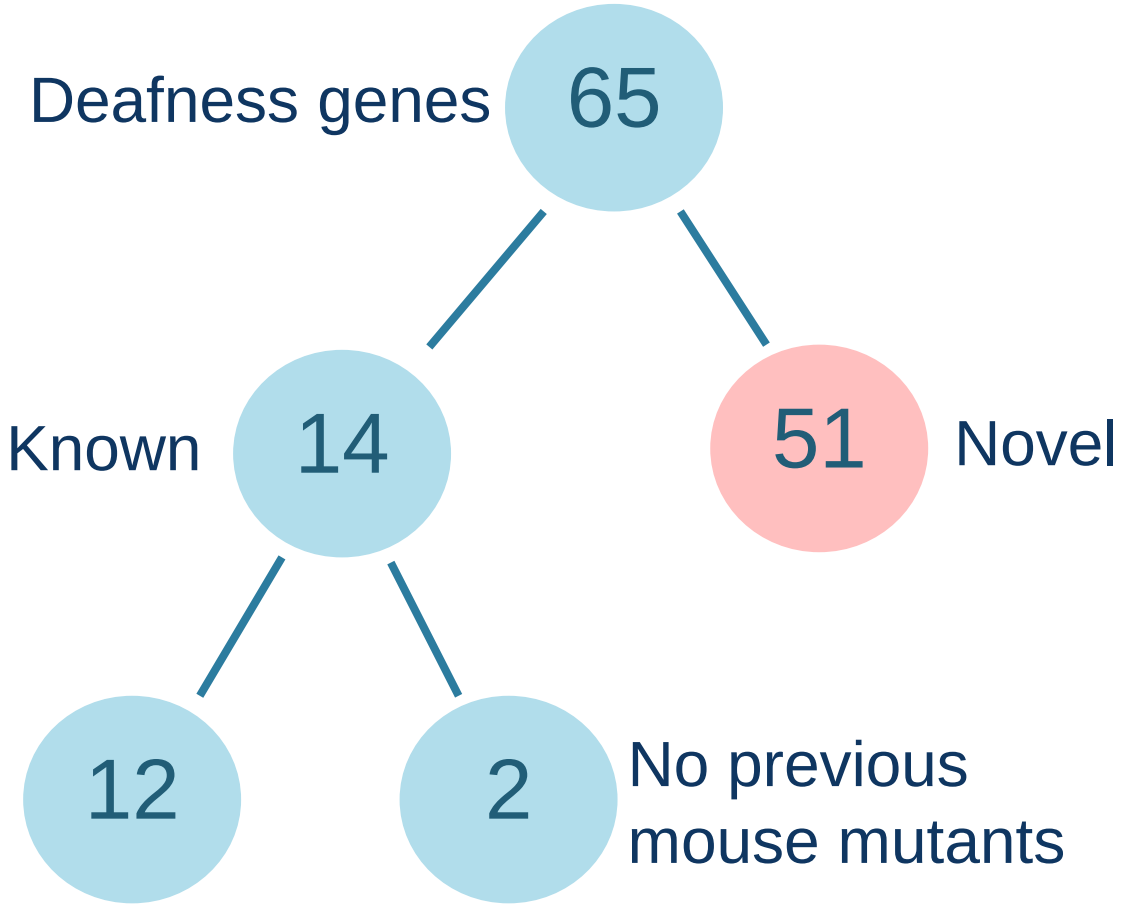


Arap1$^{-/-}$ progressive photoreceptor degeneration in mice, similar to retinitis pigmentosa in humans.

ßgal co-localizes with glutamine synthetase in retinal Müller glia



Phenotype Survey: Deafness



Adgrv1
Clrn1
Elmod1
Gipc3
Ildr1
Marveld2
Myo7a
Ocm
Otoa
Srrm4
Tprn
Ush1c

Cib2
Col9a2

4933430I17Rik
Adgrb1
Afap1l2
Aff3
Ankrd11
Ap3s1
Ap4e1
Atg4b
Atp2b1
B020004J07Rik
Baiap2l2
Ccdc88c
Cyb5r2
Dusp7
Emb
Emx1
Eps8l1
Ewsr1
Gata2
Gga1
Gm684
Gpr50
Ikzf5
Il1r2
Klc2
Klhl18
Lsm1
Mag
Med28
Myh1
Nedd4l
Nfatc3
Nptn
Odf3l2
P2rx7
Phf6
Ppm1a
Ppp2r5d
Rasal2
Rsad1
Sema3f
Sfxn3
Tmem30b
Tram2
Ube2b
Ube2g1
Ube2w
Vti1a
Wdtc1
Zcchc14
Zfp719

M Bowl *et al*, 2017 *Nature Comms*

Enhancing availability and accessibility: Integration of KOMP2 with MMRRC

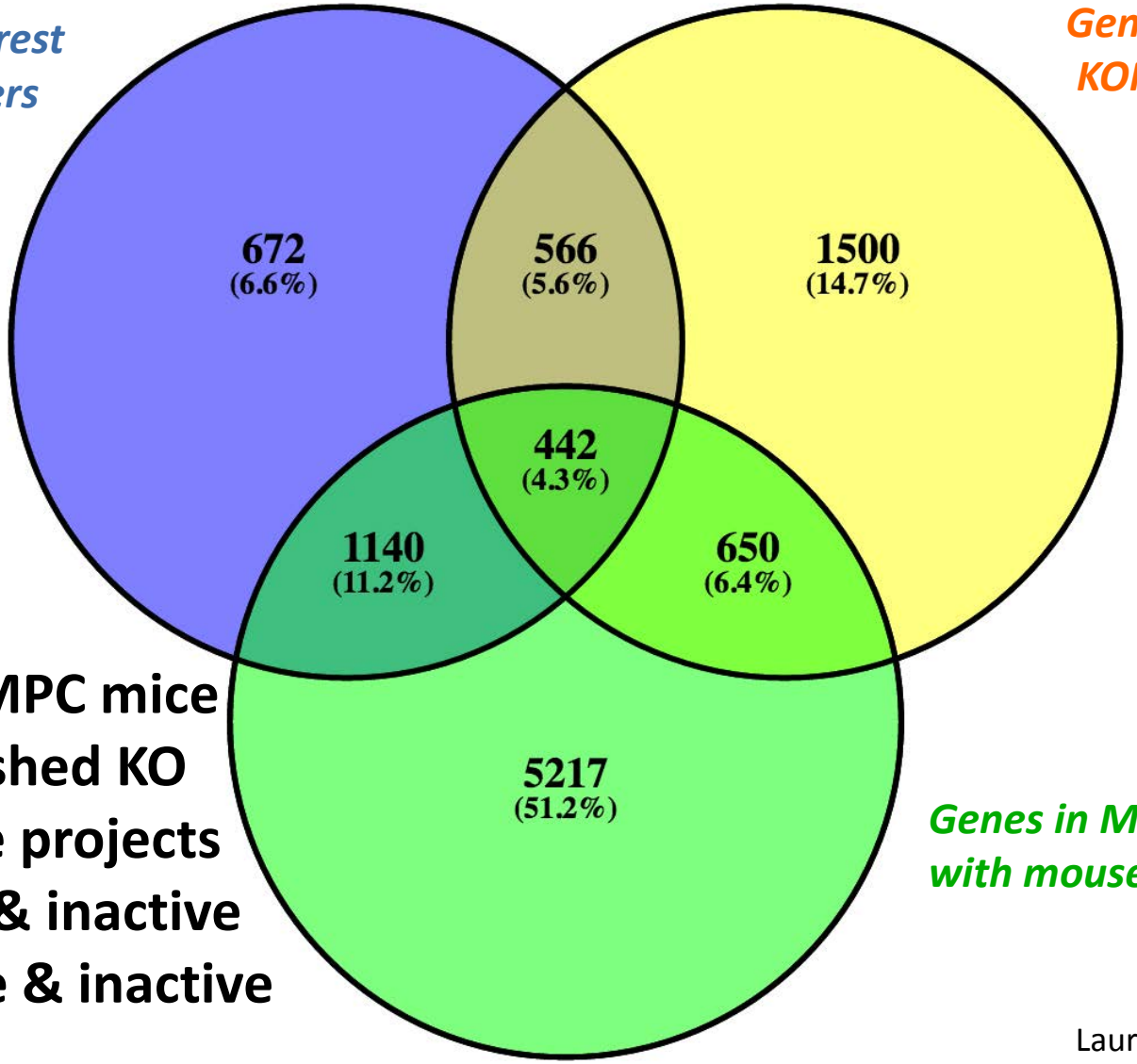
The MMRRC is a consortium of four regionally distributed archive and distribution Centers and an Informatics, Coordination and Service Center (ICSC) functioning as a fully integrated repository system.

The Centers import, quality control, maintain, archive, and distribute mouse lines upon request.

The ICSC provides an online searchable catalogue, dynamic website, strain curation services, data resources, technical assistance, and outreach and education activities.

MMRRC
Mutant Mouse Resource & Research Centers
supported by NIH

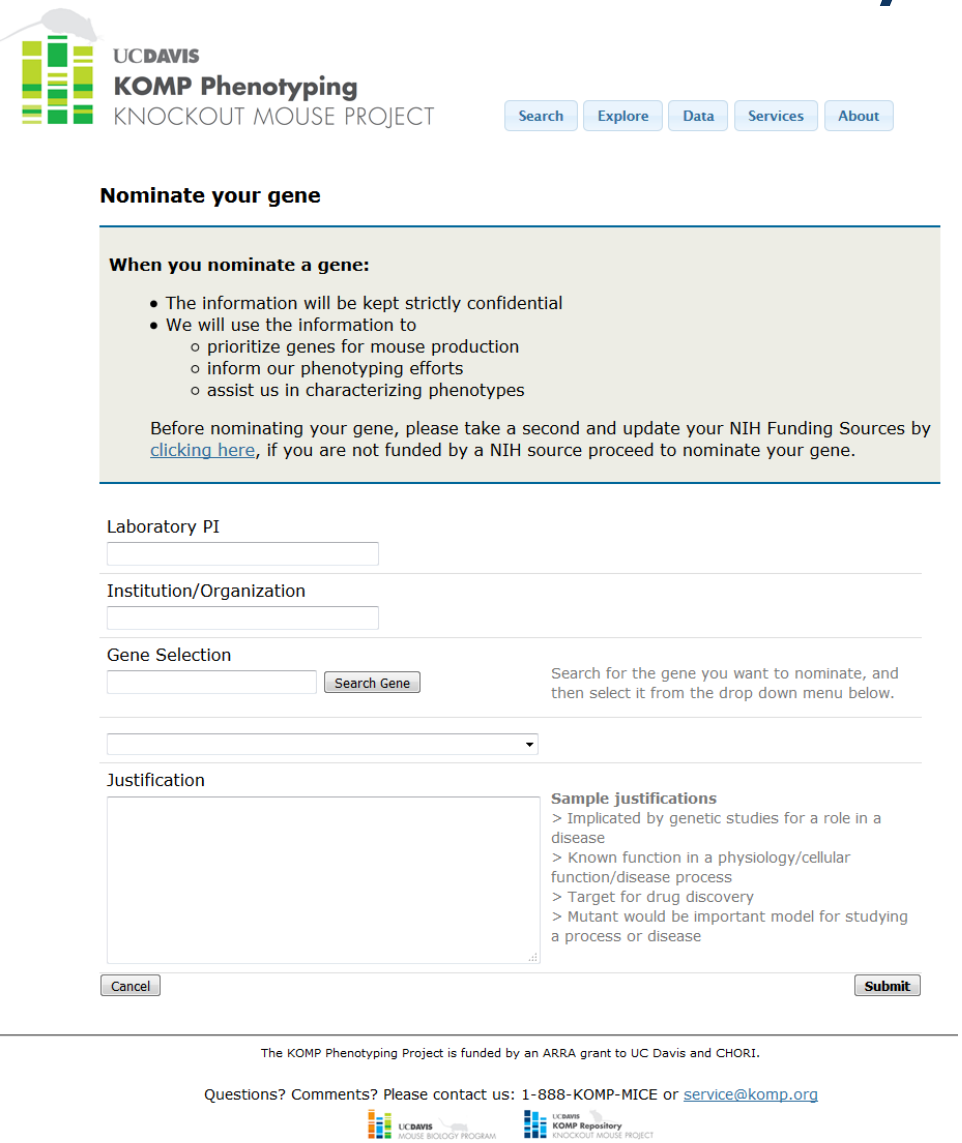
Responding to the Community

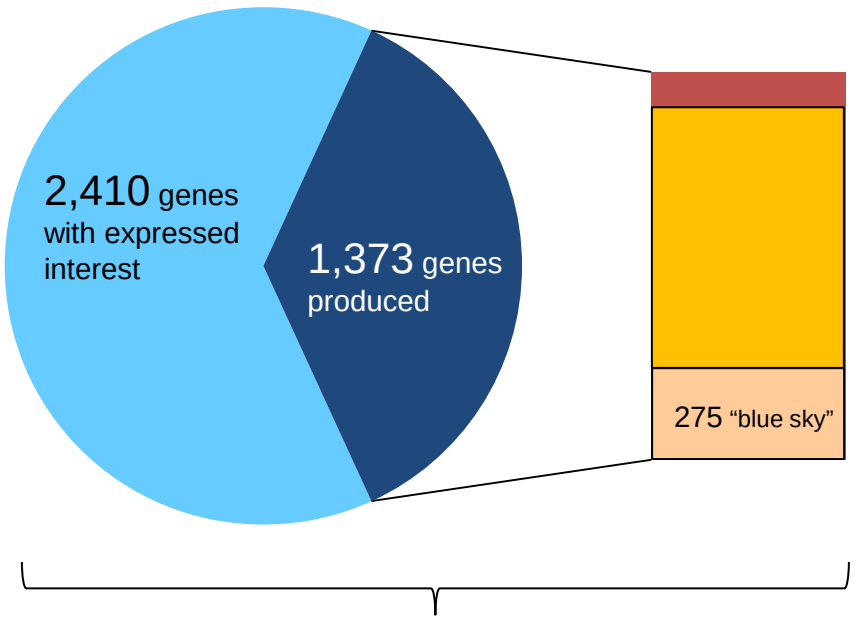


2,820 genes, no IMPC mice
1,582 (56%) published KO
1,008 (36%) active projects
1,140 (40%) mice & inactive
672 (24%) no mice & inactive

Lauryl Nutter

Community Engagement

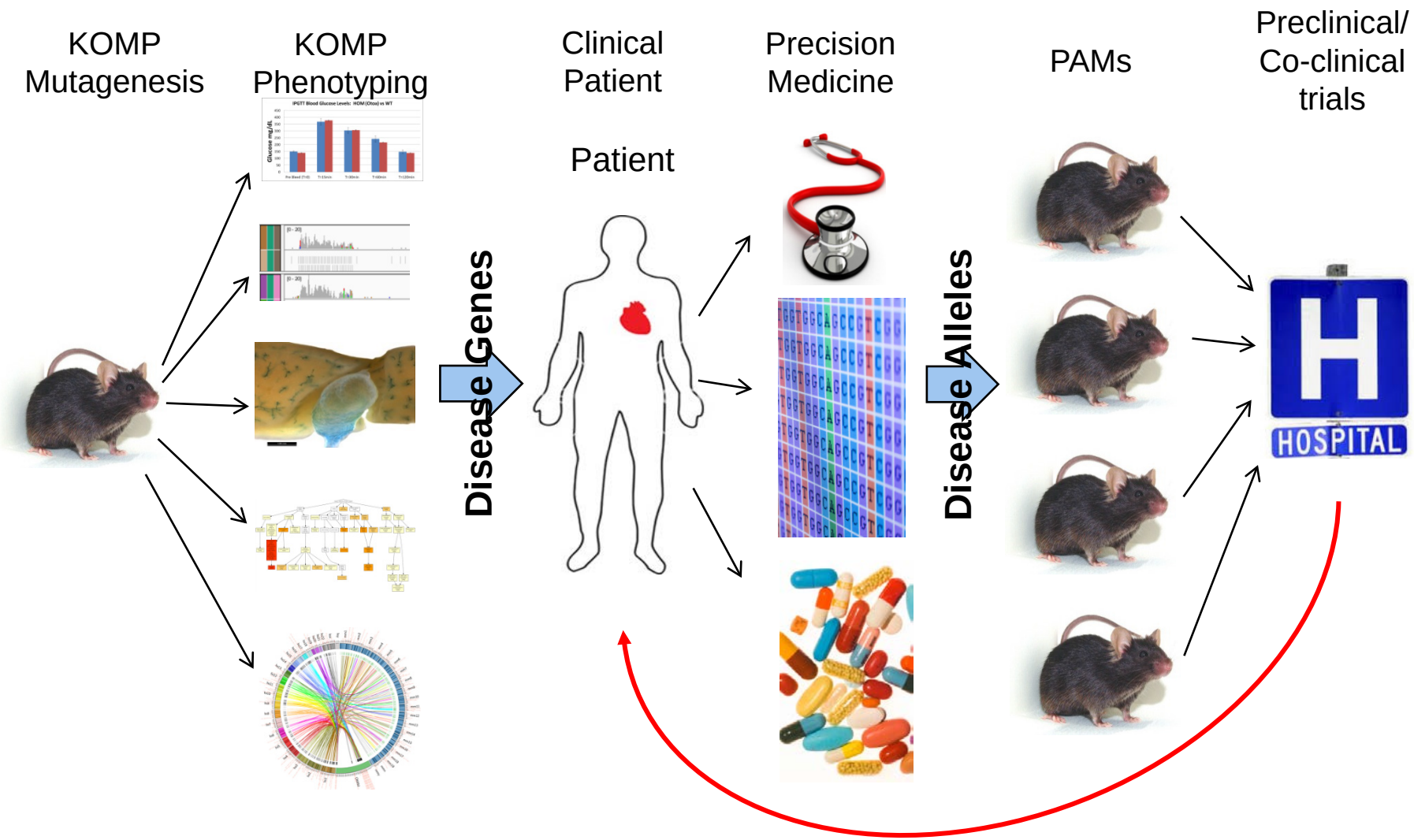




~90% of eligible genes produced were enrolled by request

Non-enrolled genes referred to MBP for contracted production

How KOMP2 informs precision mouse models



KOMP2: To what end?

- Novel insights into gene function
- Extensive new collection of disease models and new candidate disease genes
- Pervasive sexual dimorphism and pleiotropy
- Identification of new gene and phenotype relationships to elicit novel biological mechanisms
- Insights into human disease from the analysis of mouse lethal (essential) genes
- Establish fundamental genomic knowledge necessary to practice precision medicine*

**But current revised plan will leave out ~half of genome*

KOMP2 Principles and Practices

- Formulate hypothesis-generating projects
- Enable availability and accessibility of mouse lines and data to academic and commercial communities worldwide
- Encourage use of extant, rather than recreating, lines
- Maximizing awareness of lines and data
- Extend technical support services to researchers
- Engage research community in project
- Practice scientific rigor and reproducibility
- Ensure protection, preservation, and perpetuity of resource

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Acknowledgements

National Institutes of Health (USA)

Toronto Centre for Phenogenomics (Canada)

Medical Research Council & MRC Harwell (UK)

The Wellcome Trust Sanger Institute (UK)

Wellcome Trust

Helmholtz Zentrum Munich (Germany)

Institute Clinique de la Souris (France)

UC Davis

European Bioinformatics Institute

The Jackson Laboratory

Children's Hospital Oakland Research Institute

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European Commission (EU)

Infrafrontier (EU)

Australian Phenomics Network (Australia)

RIKEN BioResource Center (Japan)

Genome Canada

Model Animal Research Center (Nanjing)

Baylor College of Medicine

Charles River Laboratories

Korea Mouse Phenotyping Center

Universitat Autònoma de Barcelona