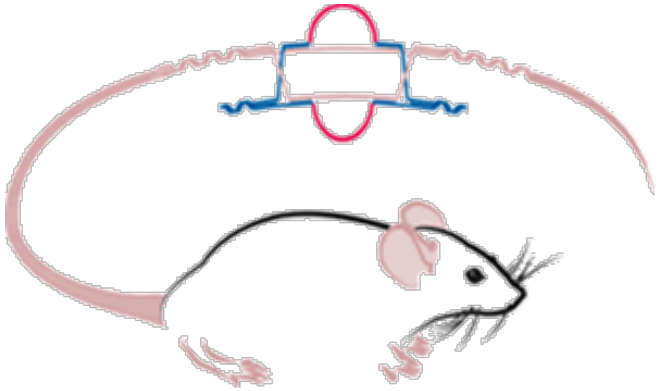


Knockout Mouse Project (KOMP) and Knockout Mouse Production and Phenotyping (KOMP^2)

Council of Councils
May 2022

Colin Fletcher, NHGRI, Oleg Mirochnitchenko, ORIP, Trish Labosky, Common Fund
and the NIH KOMP Working Group

Envisioning KOMP - 2003

The concept of a genome-wide gene knockout project was discussed at a community meeting in the fall of 2003 at the CSHL Banbury Center.

"**Mouse Genome-wide Targeted Mutagenesis**" Sept 30 - Oct 1, 2003

Low throughput – high cost and technical challenges slow production of gene knockouts (500/yr = 40 years)
Low availability – few strains deposited in public repositories
Low coverage – phenotyping is not comprehensive

"A coordinated project to systematically knock out all mouse genes is likely to be of enormous benefit to the research community"

Austin *et al*., Nature Genetics, 2004 Auwerx *et al*., Nature Genetics, 2004

Implementing the vision

1) *A comprehensive genome-wide resource of mutant ES cell lines …*
most known mouse genes could be knocked out in ES cells within 5 years

KOMP was launched in 2006 as a trans-NIH Program

$56.6 million over 5 years from the ICs
Created 8,500 ES cell lines in C57BL/6N
Alleles are nulls or conditional-ready, contain reporter

2) *ES cells should be converted into mice at a rate consistent with project funding and the ability of the worldwide scientific community to analyze them … and phenotyped by a limited set of broad and cost-effective screens*

KOMP2 launched in 2011 as a trans-NIH & Common Fund Program

$225 million over 10 years
Created 5,500 mouse lines (2,500 ES/3,000 CRISPR)
Broad phenotyping across many domains

3) *All ES cell clones and mice (as frozen embryos or sperm) should be available to any researcher at minimal cost*

KOMP reagents are distributed by the MMRRC network

4) *All mouse phenotyping and reporter expression data should be deposited into a public database.*

KOMP web portal operated by European Bioinformatics Institute (EBI)

KOMP and International Partners

Leveraging international partnerships has doubled production

2006 - 2011

IKMC – The International Knockout Mouse Consortium

KOMP + European Conditional Mouse Mutagenesis Programme

17,500 knockouts as ES cell clones

2011 - present

IMPC – The International Mouse Phenotyping Consortium

KOMP2 + many international partners

10,013 mouse strains produced to date

8,782 phenotyped to date

IMPC

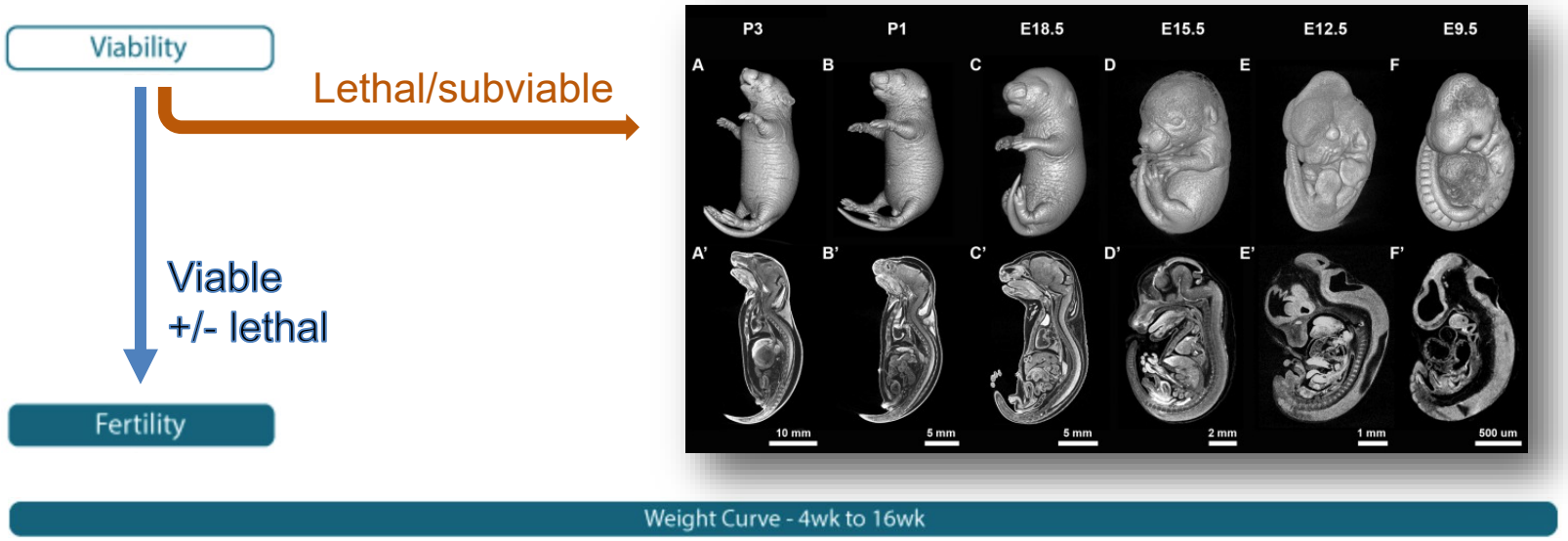
International Mouse Phenotyping Consortium

22 currently active members

www.mousephenotype.org

Implementation

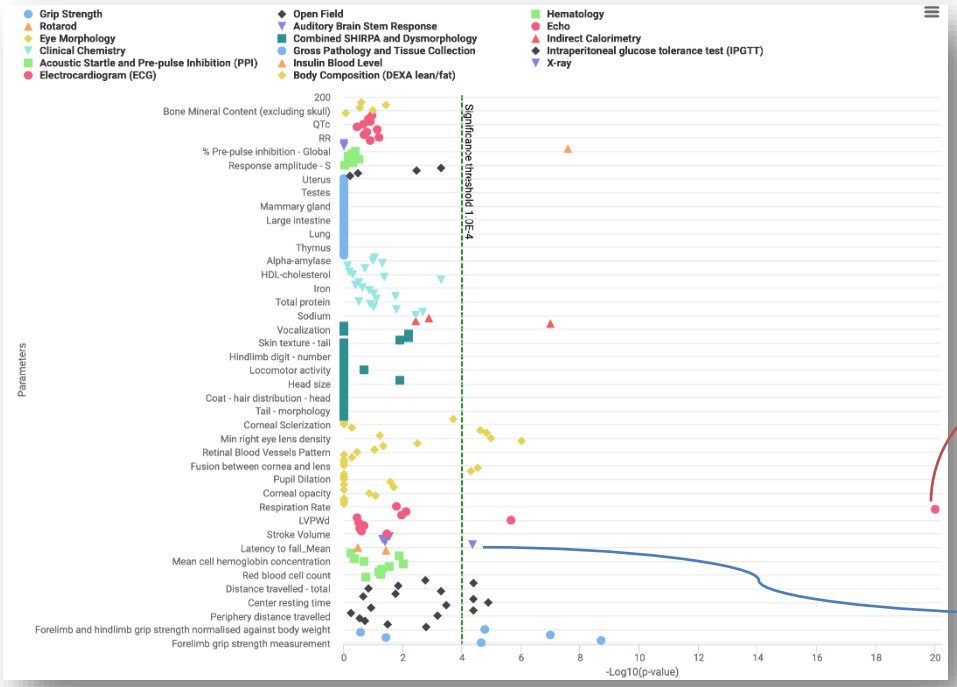
International Mouse Phenotyping Consortium (IMPC) partners have implemented a standardized phenotyping pipeline that interrogates multiple biological domains. Data is uploaded into a common archive for analysis, annotation, and dissemination. Mice are deposited in publicly funded repositories.

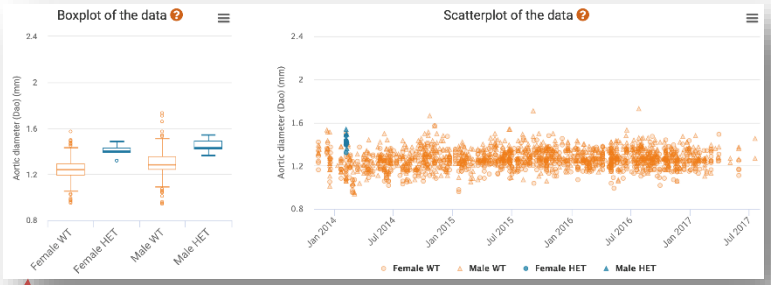


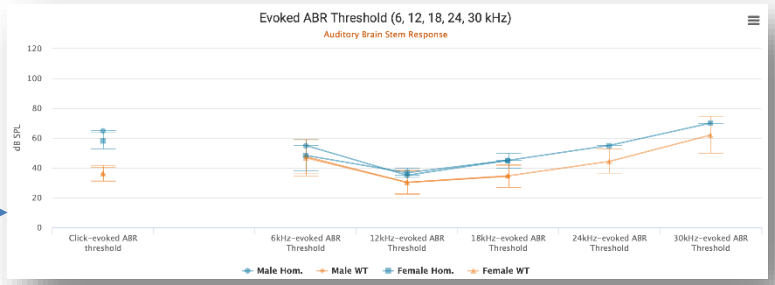
Dissemination

Name LSM1 homolog, mRNA degradation associated
MGI ID MGI:1914457
Synonyms 2810025O06Rik U6 small nuclear RNA associated
Viability Homozygous - Subviable
Embryo viewer N/A
Other links MGI Ensembl

Ideogram of test results





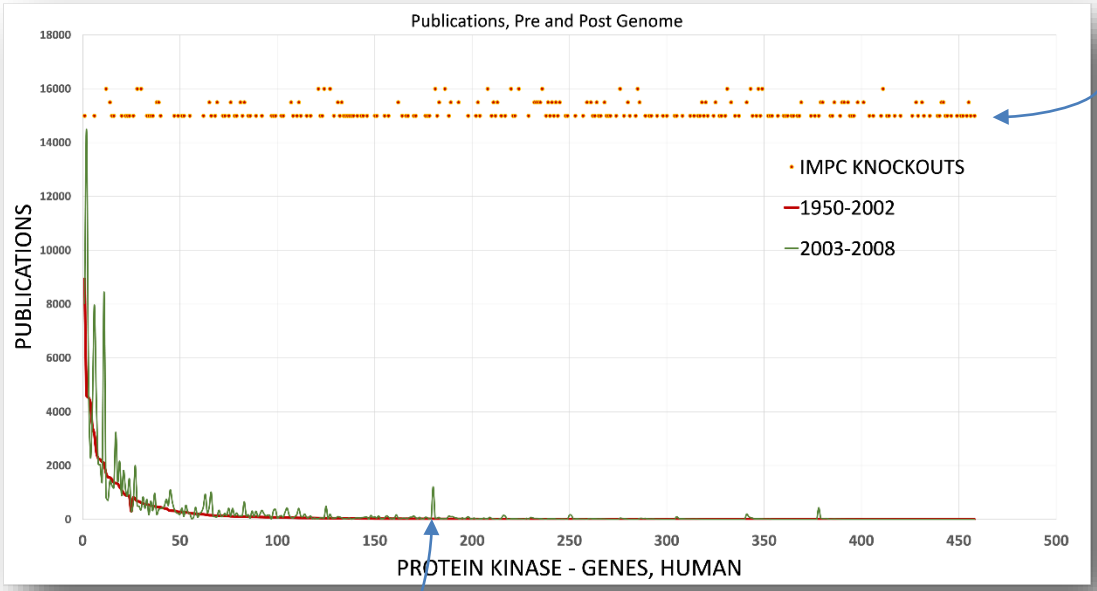


Interactive Manhattan plot (left) provides overview of quantitative data and links to each assay result (right)

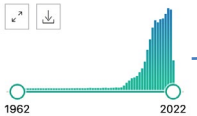
Benefits

KOMP empowers research by providing hypotheses and tools:
novel gene – phenotype connections
access to the dark protein coding genes

Edwards et al., Distribution of publications has not changed between pre and post genome
- research activity remains focused on known genes

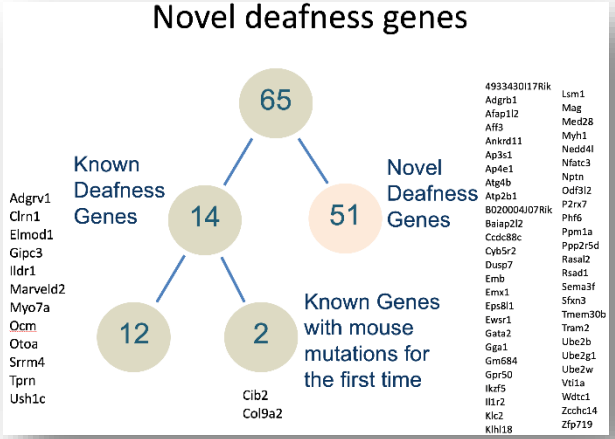


KO coverage extends across entire protein kinase family



Yup, that's BRAF

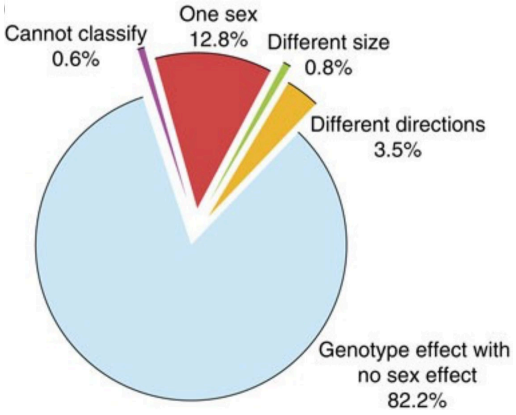
IMPC Deafness screen identifies many novel genes

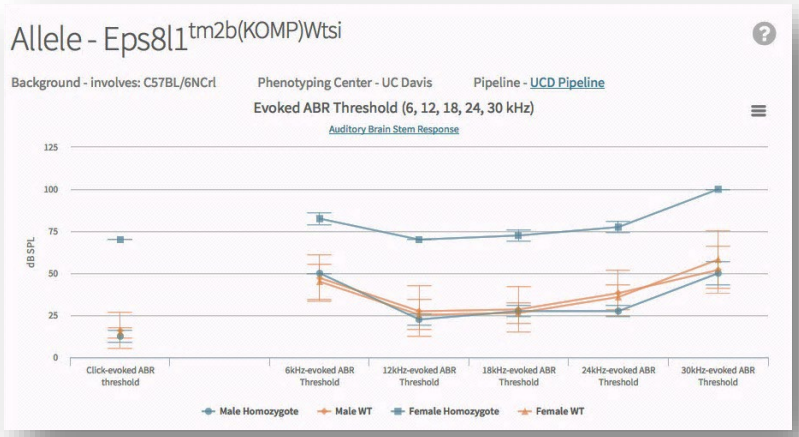


Bowl et al Nature Comms

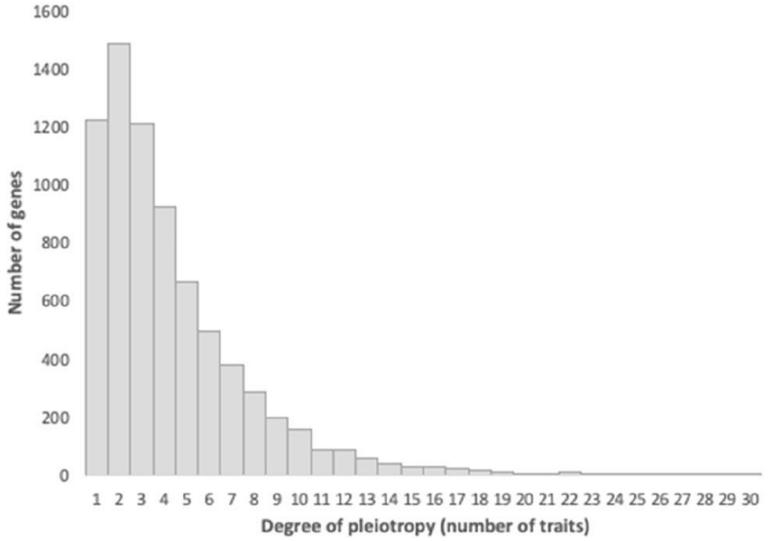
Benefits

KOMP provides insight into biology:
pleiotropy
sex as a biological variable

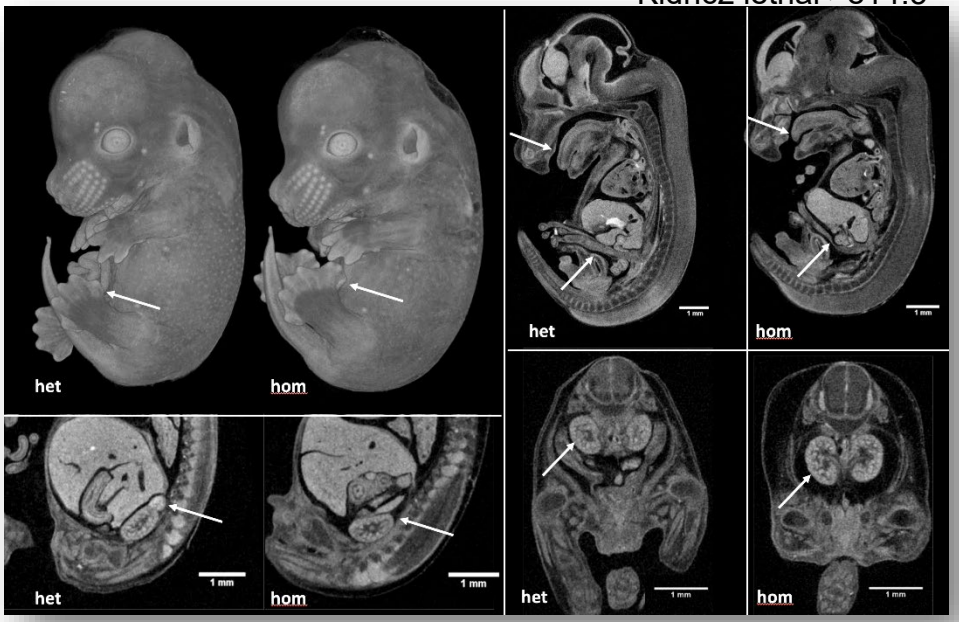




Effect of sex as a biological variable



Kldhc2 lethal >e14.5



Pleiotropy is commonly observed

Karp et al Nature Comms

Munoz-Fuentes et al Mamm Genome

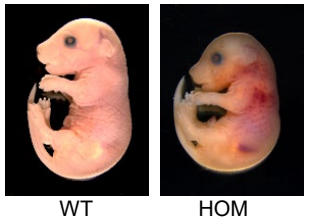
Cdc42bpb in congenital diaphragmatic hernia

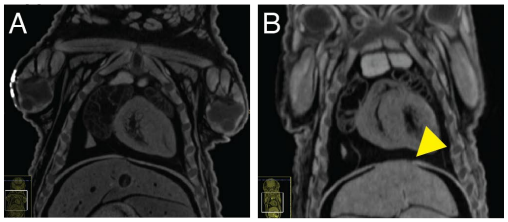
A human disease gene candidate

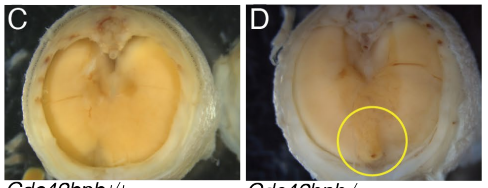
- Missense and stop-gained alleles nominated by Dr. Wendy Chung as part of CF-supported supplement (UM1 OD023222-09-S3)
- KO was already in the JAX KOMP2 pipeline
- Subviable at E18.5 with minor gross morphology defects noted in some individuals
- Imaging, however revealed evidence of diaphragmatic hernia in E18.5 embryos
- Additional analysis confirmed a highly penetrant ventral hernia in mutants
- With these data, the investigator had sufficient evidence to move forward

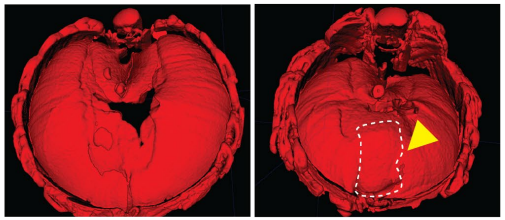
KOMP Pipeline

	HOM	HET	WT
Wean	1 (0.9%)	71 (68%)	41 (32%)
E18.5	5 (5.6%)	47 (52%)	38 (42%)



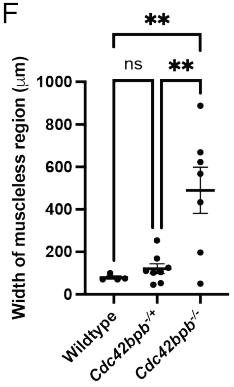






E

	Hernias found (% penetrance)
WT	0/15 (0%)
HET	2/33 (6%)
HOM	6/8 (75%)

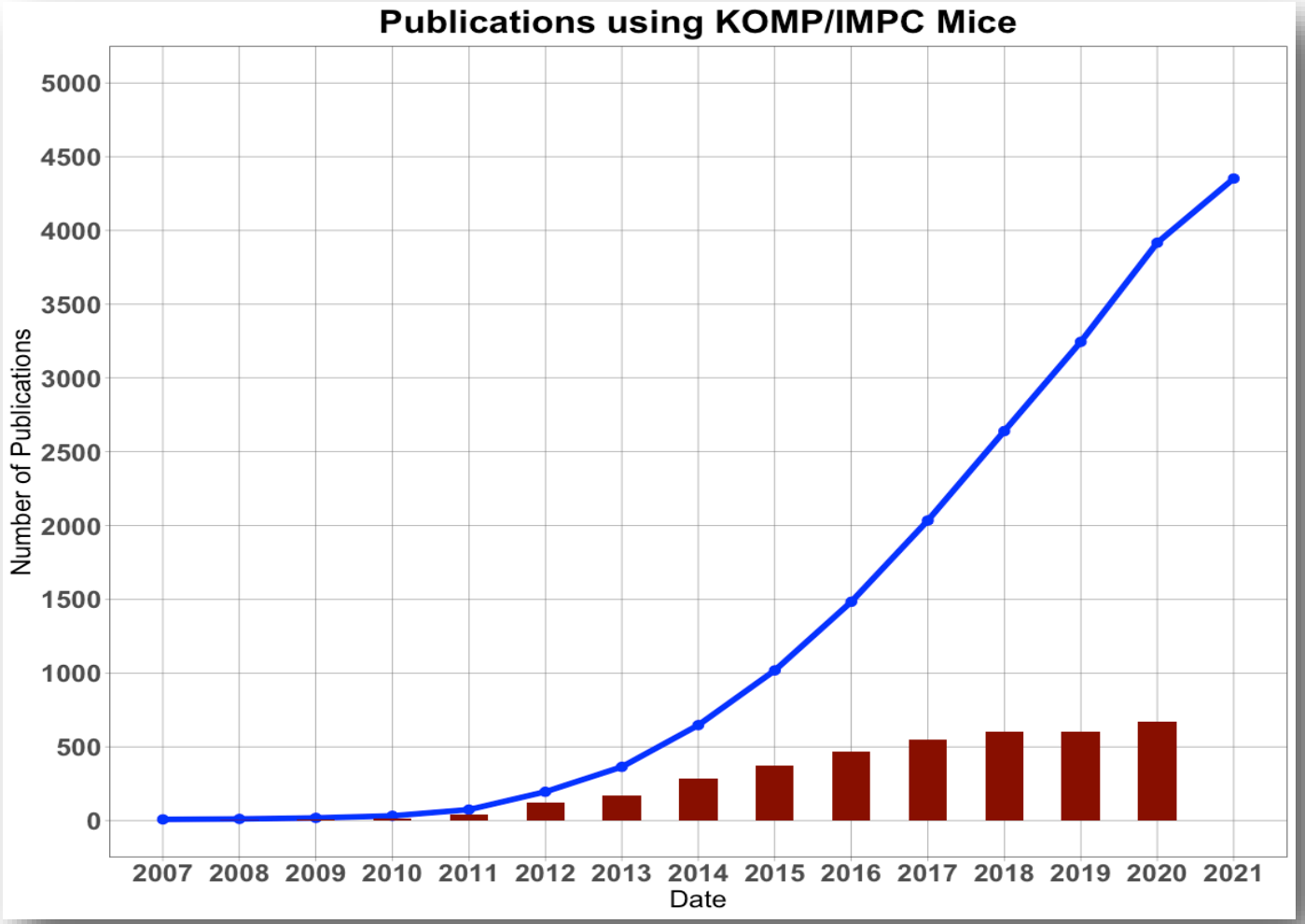


Gabriella Miller Kids First PEDIATRIC RESEARCH PROGRAM Data Resource Center

Uptake

The research community is purchasing and using KOMP/IMPC mice

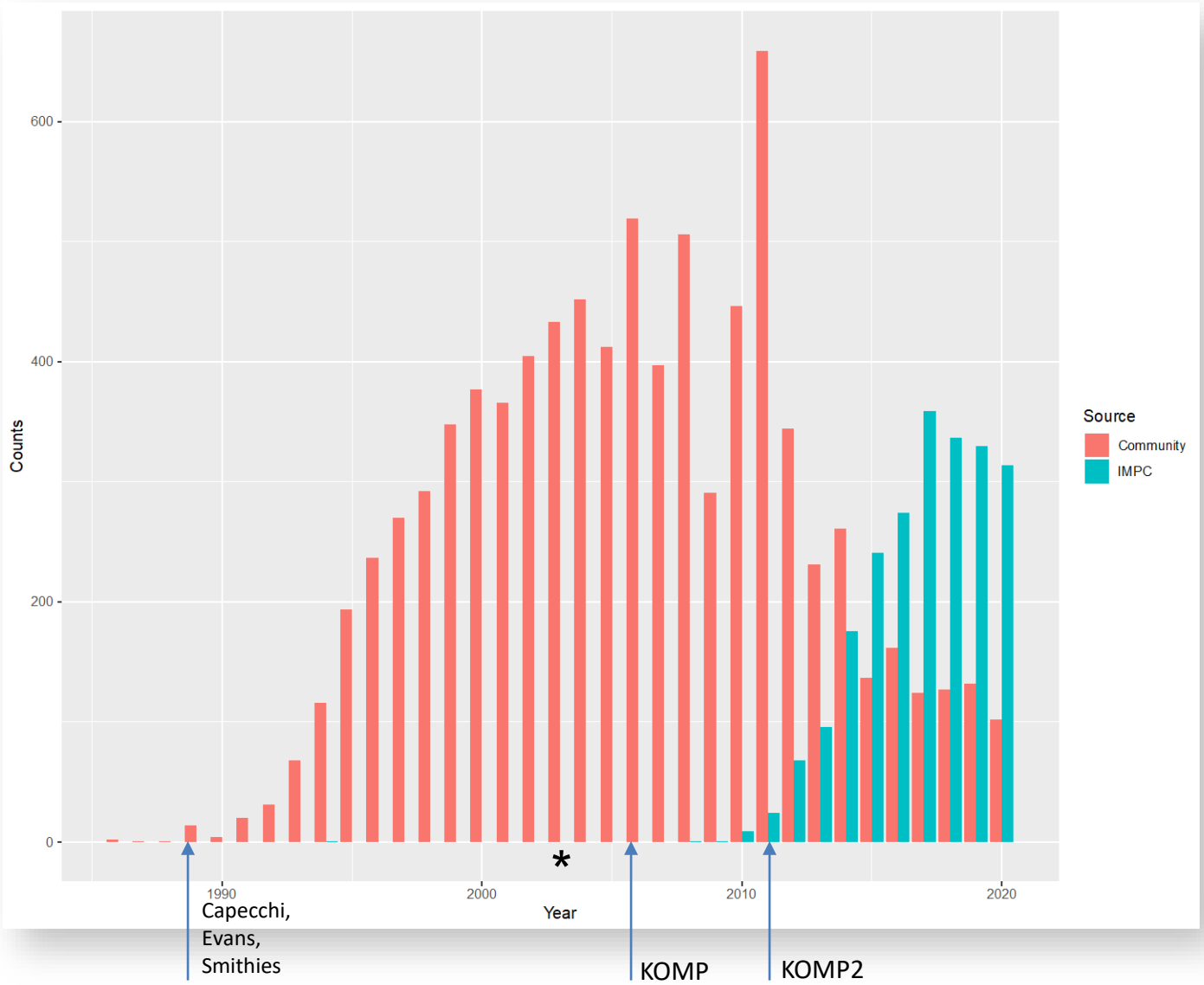
Admin IC	Count
NIDDK	659
NCI	649
NIGMS	612
NHLBI	482
NIAID	397
NINDS	385
NICHD	308
OD	253
NHGRI	235
NIAMS	213
NIA	199
NEI	191
NCRR	181
NCATS	127
NIMH	118
NIDA	89
NIDCD	80
NIDCR	76
NIEHS	69
NIAAA	44
NCCIH	21
NIMHD	17
NIBIB	16



4,442 Publications using IMPC reagents

Uptake

Publications that report a new knockout strain.



Red Bars:
KO made in a lab

Teal Bars:
KO from IMPC

Analysis shows shift away from lab-made knockouts. Publications now show preferential use of IMPC alleles

* Banbury

Kevin Peterson, Jax

Accomplishments and Plans

"A coordinated project to systematically knock out all mouse genes is likely to be of enormous benefit to the research community"

- Created over 10,000 knockout lines, close to 9,000 have completed phenotyping
- Discovered extensive pleiotropy and sexual dimorphism
- Deduced that one third of genes have embryo defects and are enriched for human disease genes.
- Created hundreds of novel mouse models of human disease, based on phenotype match with clinical features.
- Published systemic analyses on
  - Metabolism
  - Eyesight
  - Hearing
  - Bone metabolism
  - Cardiovascular function

Provided resources that have enabled >4,400 peer-reviewed publications

- Aim to complete 3,000 more KOs in the next 5 years

  ~1,000 will be trans-NIH funded

Questions/Discussion