Canine Cancer Genomics

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Dogs in Genetics

- 78.2 million dogs owned in U.S.
  - ~35% of 131 million U.S. households have dogs
- Dogs live with and are exposed to the same environment as their human owners
- Dogs receive preventative and diagnostic healthcare
- Dogs live to old age
- Population structure well suited to genetic studies

Cancer is the #1 Cause of Disease Associated Death in Dogs

More than 1 in 4 dogs will develop cancer in their lifetime

Many cancers show strong breed predisposition:

Cancer	Breed	Relative Risk
Gastric Carcinoma	Chow Chow	10-20
Hemangiosarcoma	Golden Retriever	12.4
Lymphoma	Boxer	4.5
Mammary Carcinoma	Doberman Pinscher	3.2
Mast Cell Tumor	Boxer	16.7
Pancreatic Carcinoma	Airedale	16.9
Osteosarcoma	Giant breeds	60.1
Bladder Cancer	Scottish Terrier	19

Canine TCC of the Bladder

- Transitional cell carcinoma of the bladder accounts for 2% of all canine tumors
- Affects up to 20,000 dogs each year
- Scottish terriers are 19 times more likely to get TCC than the average dog (Shetland sheepdogs and West Highland white terriers have a 5x increased risk)

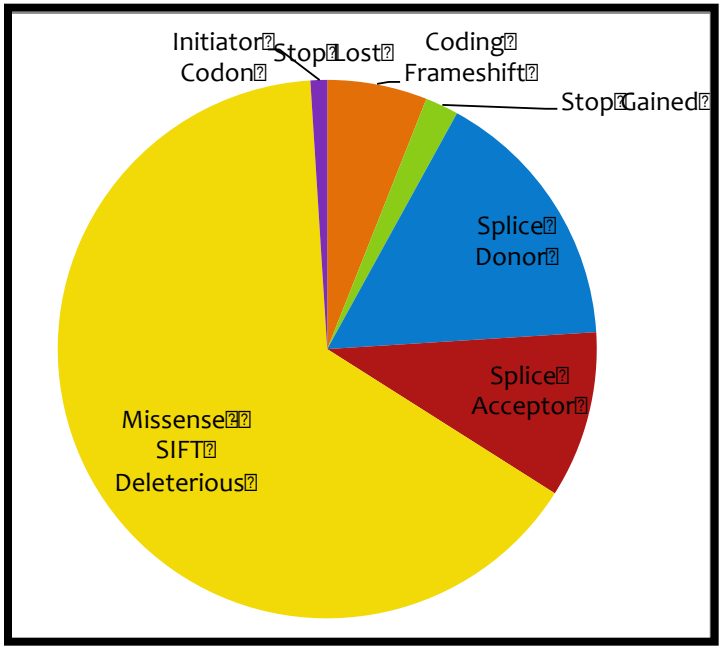
RNAseq of Canine TCC

- Survey sequence of 4 tumors (2 ST, 1 WHWT, 1 SSD), and 2 normal bladder urothelial tissues.

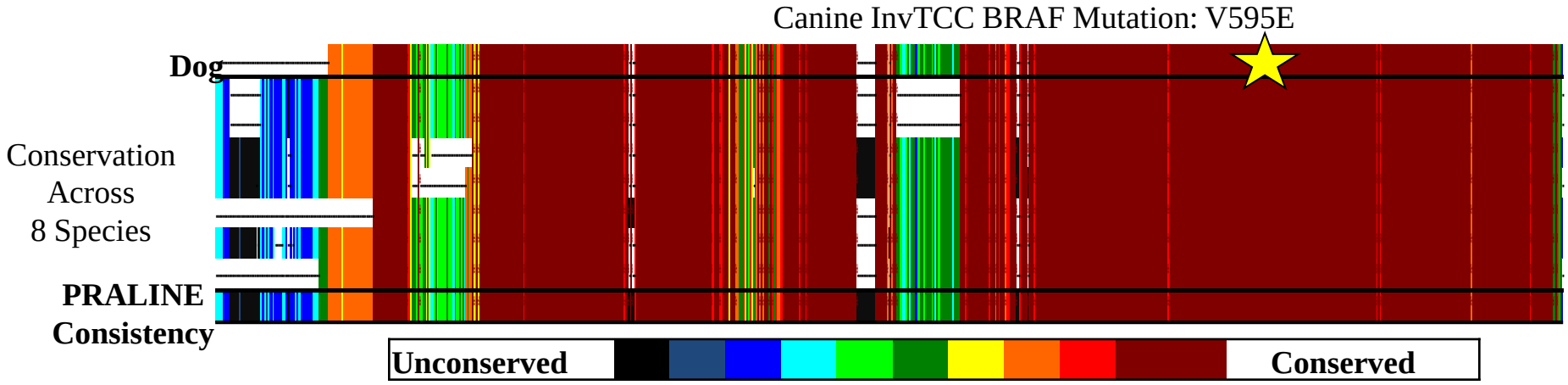
- Sequenced complete transcriptome using Illumina Hiseq with paired end libraries

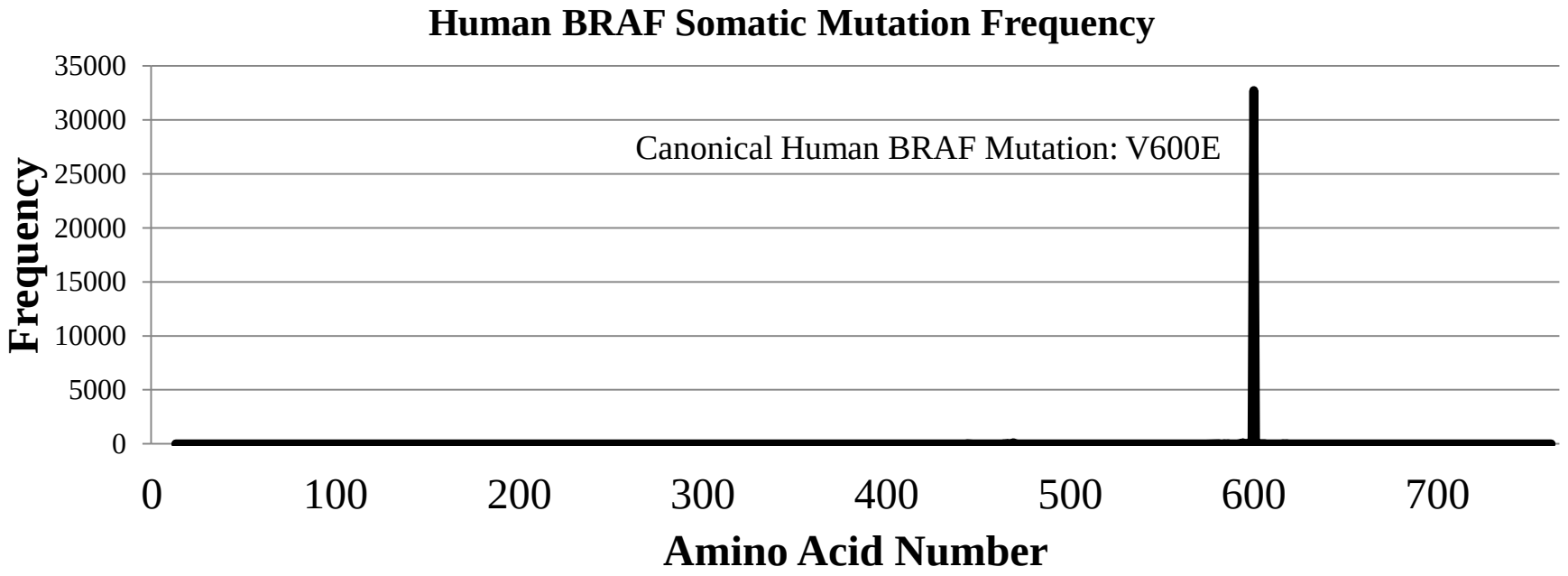
Increase information base:

- Identify transcripts that are not annotated
- Find genes that are expressed in only tumor or only normal bladders
- Identify somatic mutations

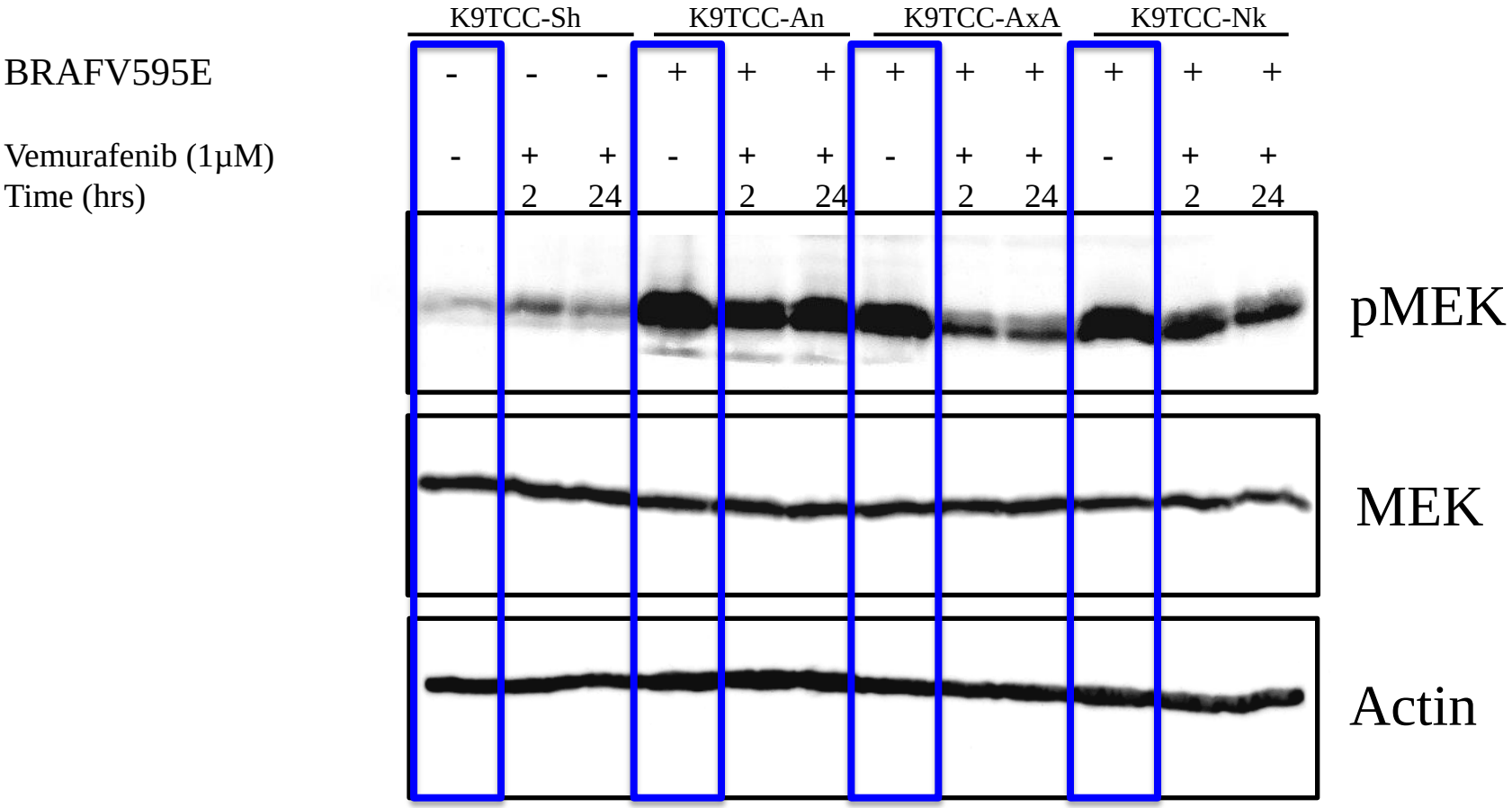


Mutation in Canine BRAF is Identical to Human BRAFV600E

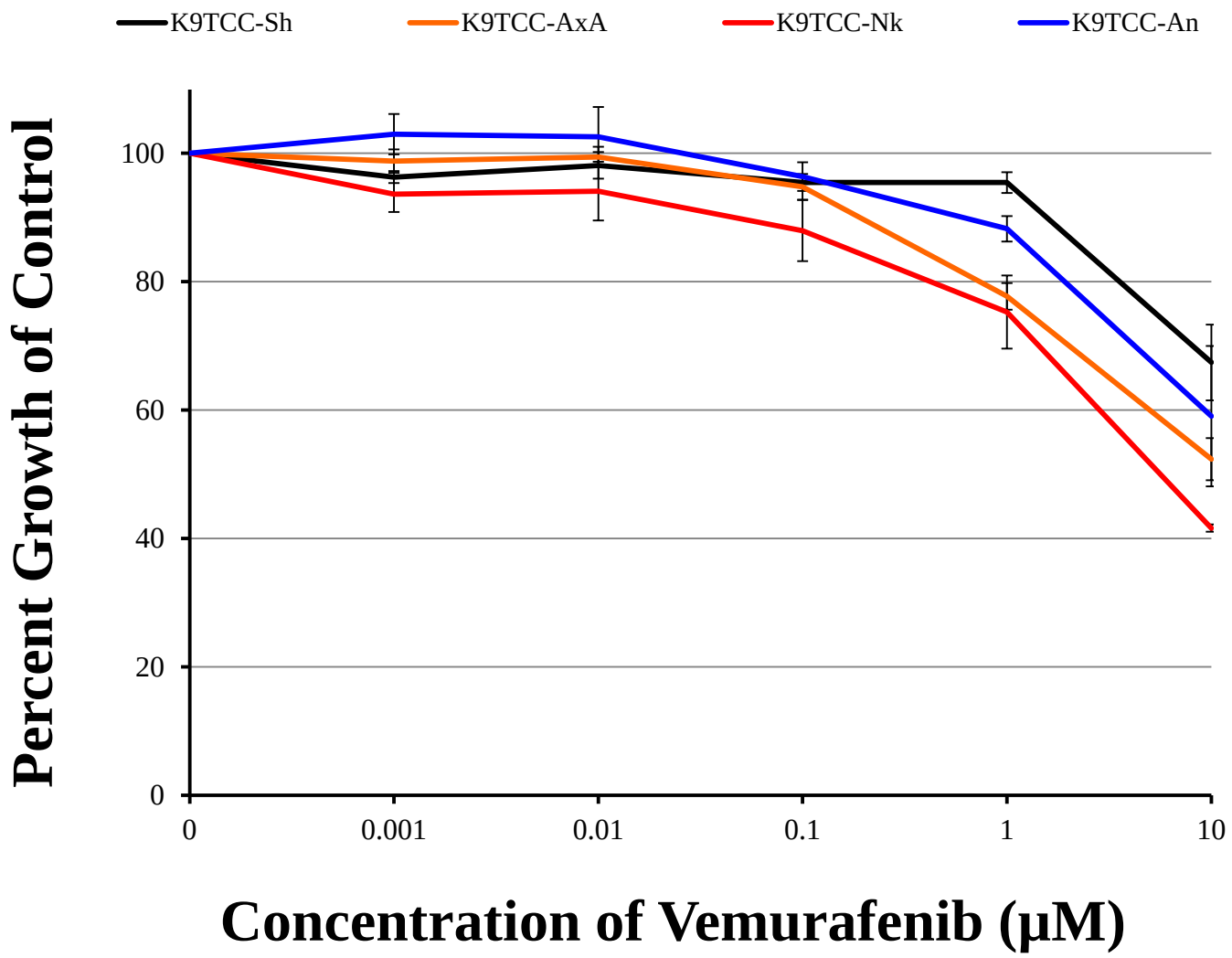




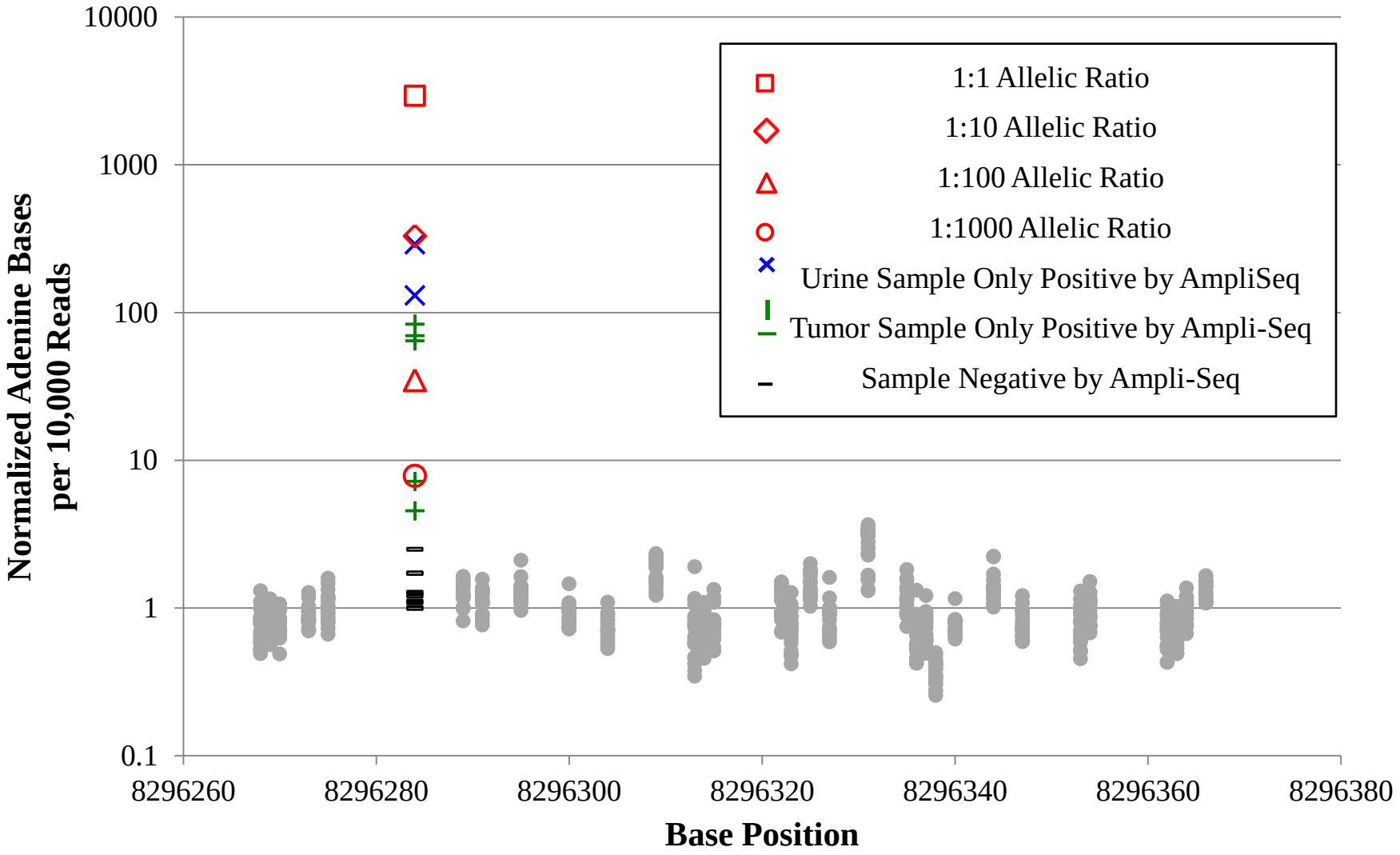
The BRAF Mutation Activates the MAPK Pathway By Phosphorylating MEK



BRAF Inhibitor Vemurafenib Reduces Cell Growth in Cells with BRAFV595E



Next Generation Sequencing Can Identify the Mutation in <1 Allele per 1000



Additional RNAseq in TCC

- Completed 22 tumors and 8 normal bladder urothelial tissues.

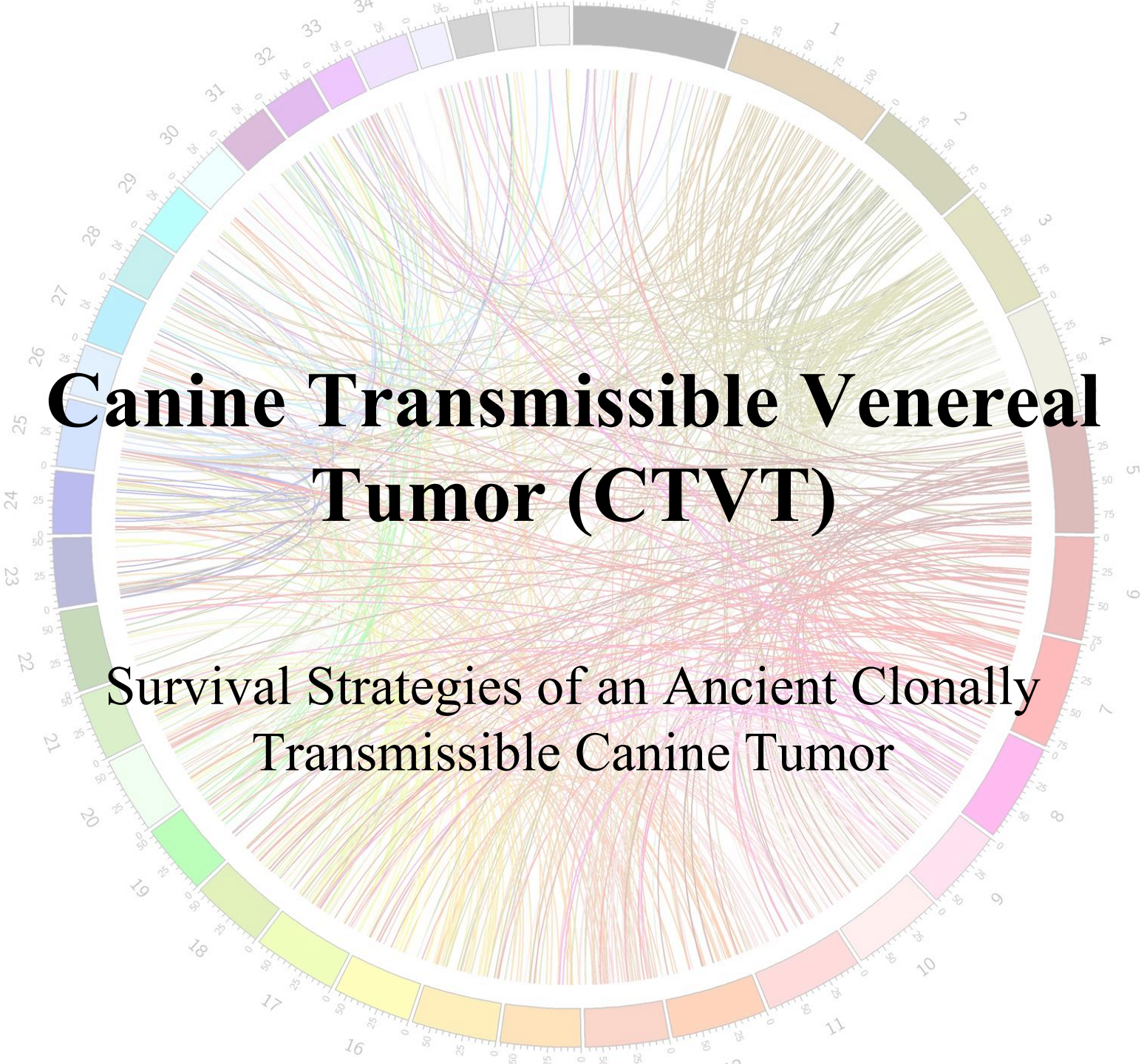
- Sequenced transcriptome using Illumina Hiseq with paired end libraries

Analyses underway:

- Identify somatic mutations: Filter out mutations found in normal tissues and the multi-breed database

- Analyze expression patterns

- Compare BRAF+ and – tumors: identify alternate drivers, additional players, compare gene set with human tumors

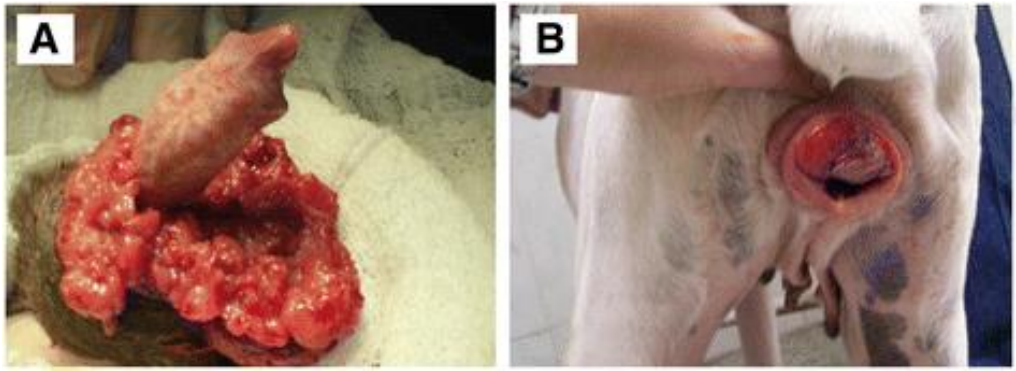


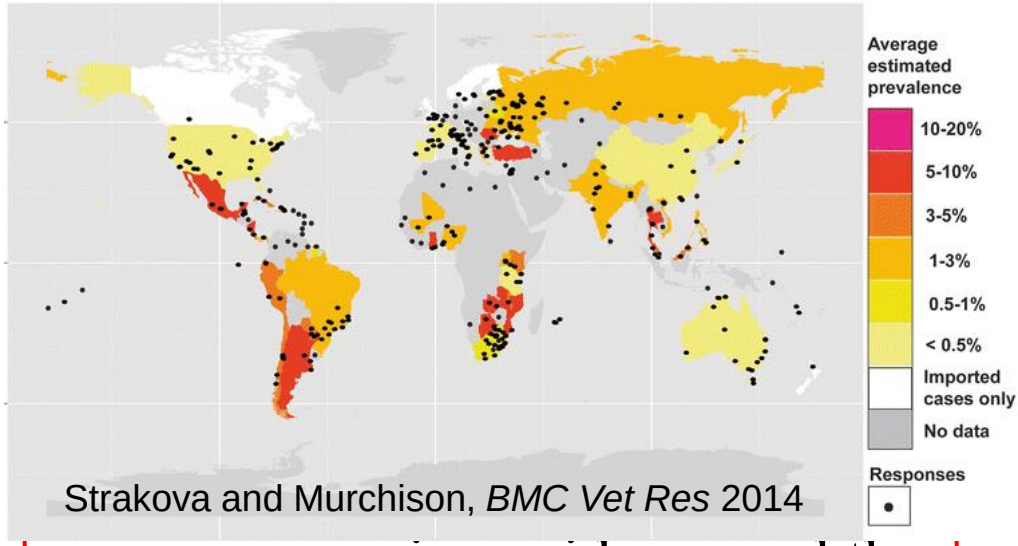
Canine Transmissible Venereal Tumor (CTVT)

Survival Strategies of an Ancient Clonally Transmissible Canine Tumor

Canine Transmissible Venereal Tumor (CTVT)

- Clonally transmissible parasite - single founder tumor spreads from dog to dog via **sexual transfer of malignant cells**
- World's oldest known continuously propagating somatic cell lineage – thousands of years, endemic across the globe
- Transmitted during months-long period of evasion of host immune defenses, later eliminated
- All CTVT tumors have **shared origins** in the single founder tumor - strong genetic identity with one another, markedly distinct from their transient host

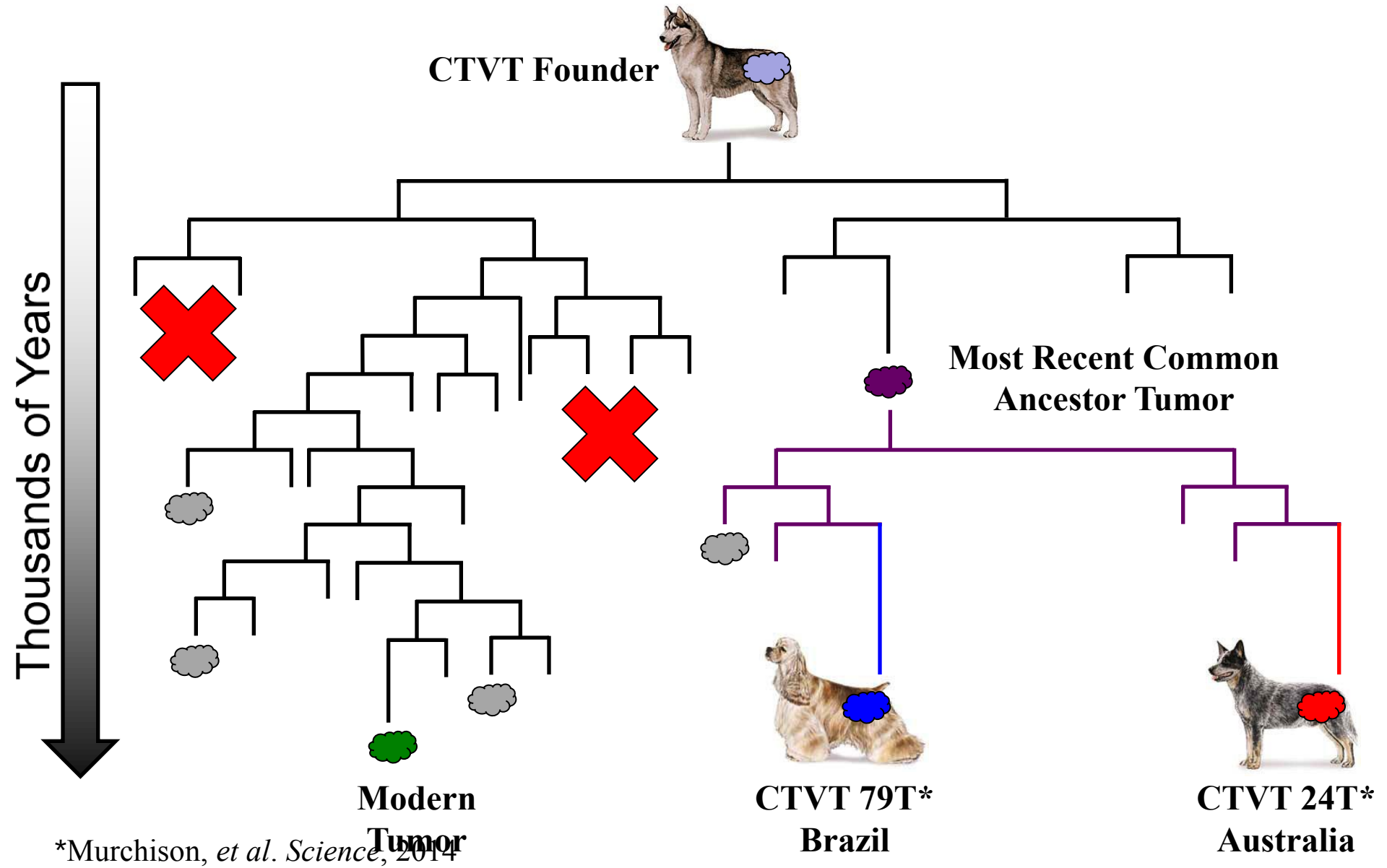




Strakova and Murchison, *BMC Vet Res* 2014

perseverance in canids around the globe?

CTVT has Propagated and Evolved for Thousands of Years



*Murchison, *et al*. *Science*, 2014

The CTVT Genome =
An Ancient Canid Genome + Somatic Mutations

Host Contamination

Inherited Alleles from Founder

Systematic Errors

Somatic Drivers of Clonal Transmissibility

Lineage-Specific Mutations

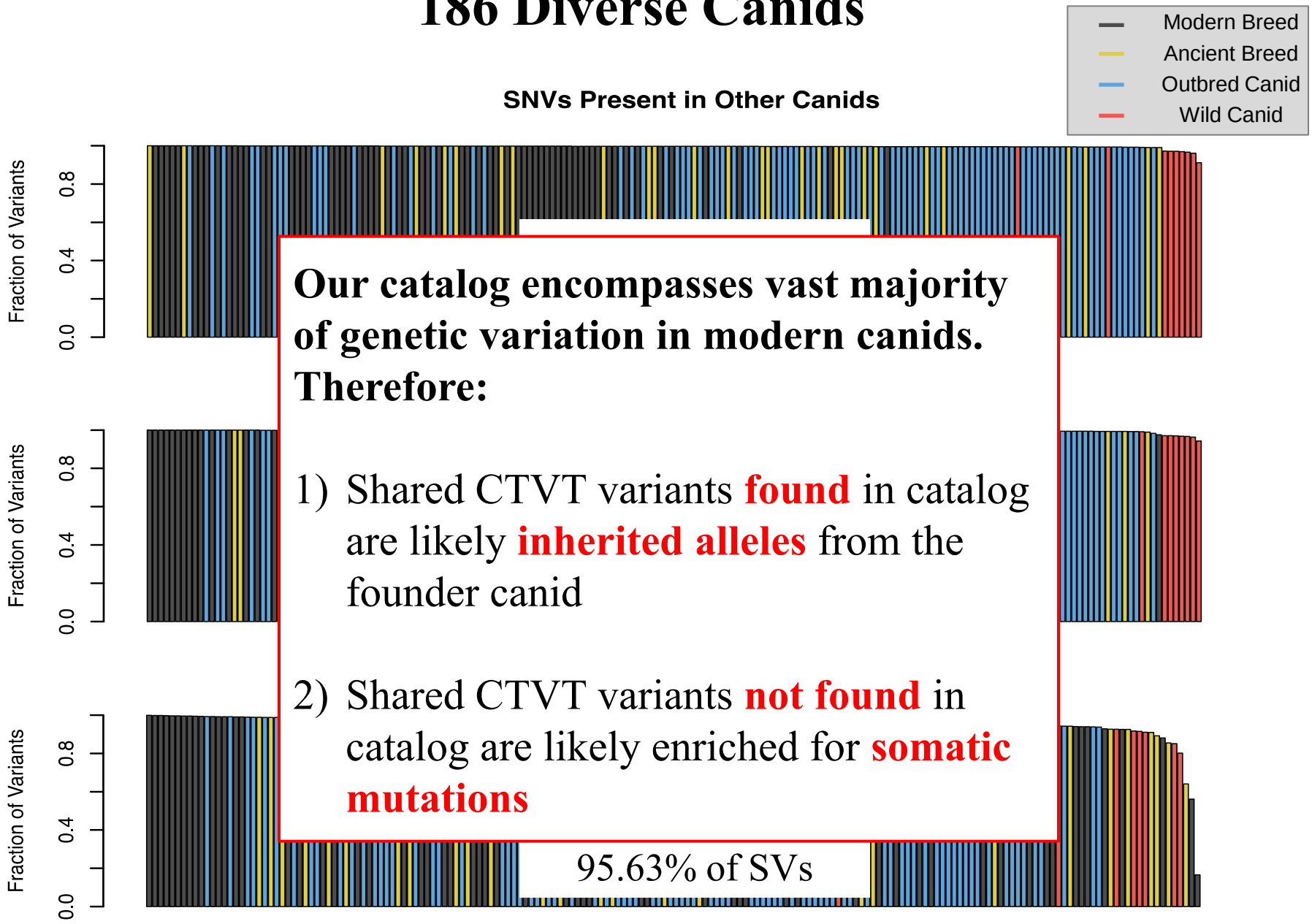
Somatic Mutations

Germline Genome of CTVT Founder Canid

CTVT 79T

CTVT 24T

Nearly All Canine Variation is Present in Panel of 186 Diverse Canids



Our catalog encompasses vast majority of genetic variation in modern canids. Therefore:

1) Shared CTVT variants **found** in catalog are likely **inherited alleles** from the founder canid

2) Shared CTVT variants **not found** in catalog are likely enriched for **somatic mutations**

Canine Variation Catalog Segregates Founder Alleles from CTVT Somatic Mutations

CTVT Founder

CTVT 79T

CTVT 24T

High-Quality Shared

Inherited Alleles from CTVT Founder

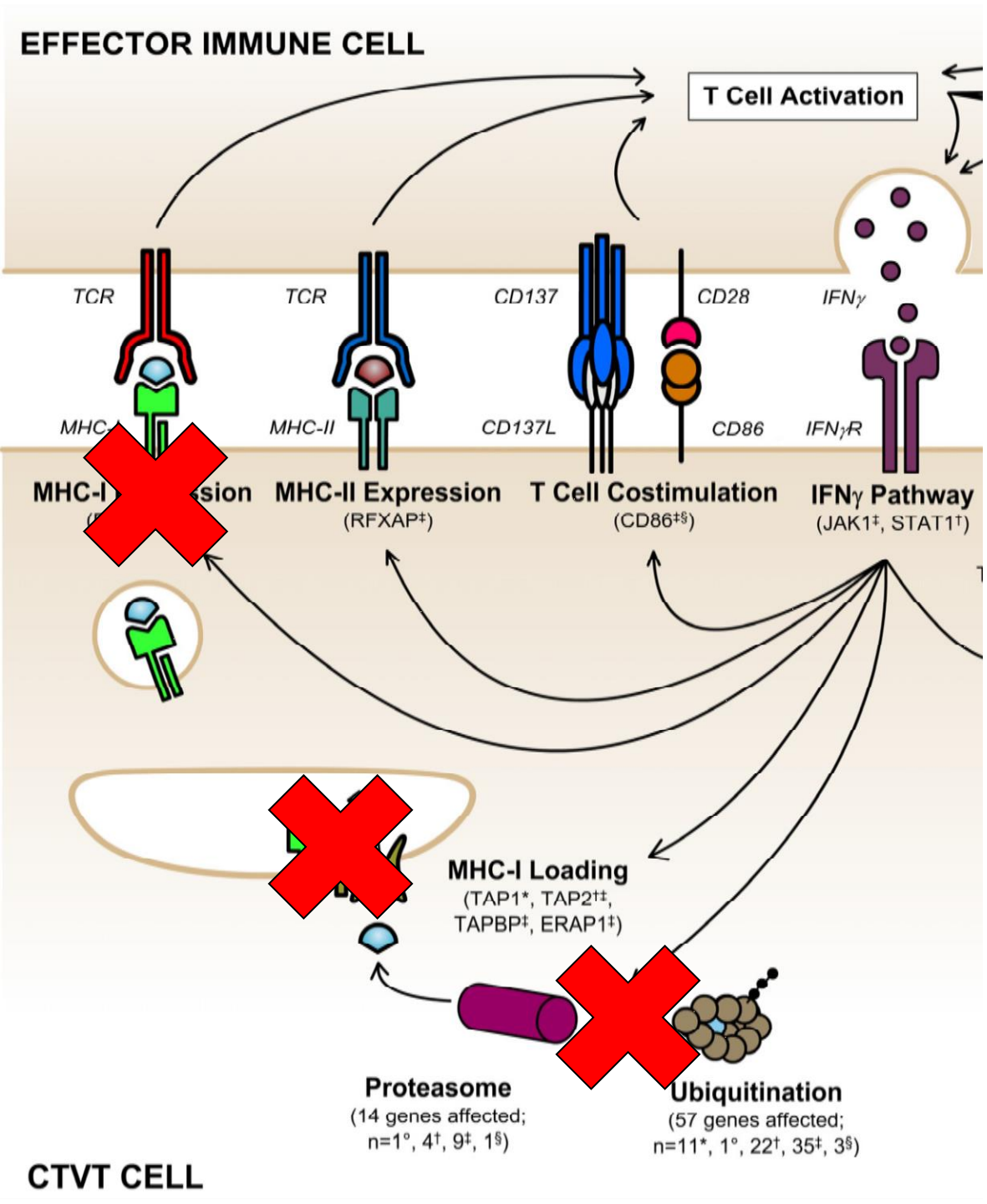
YES

Found in Ostrander Lab Canid WGS Catalog?

NO

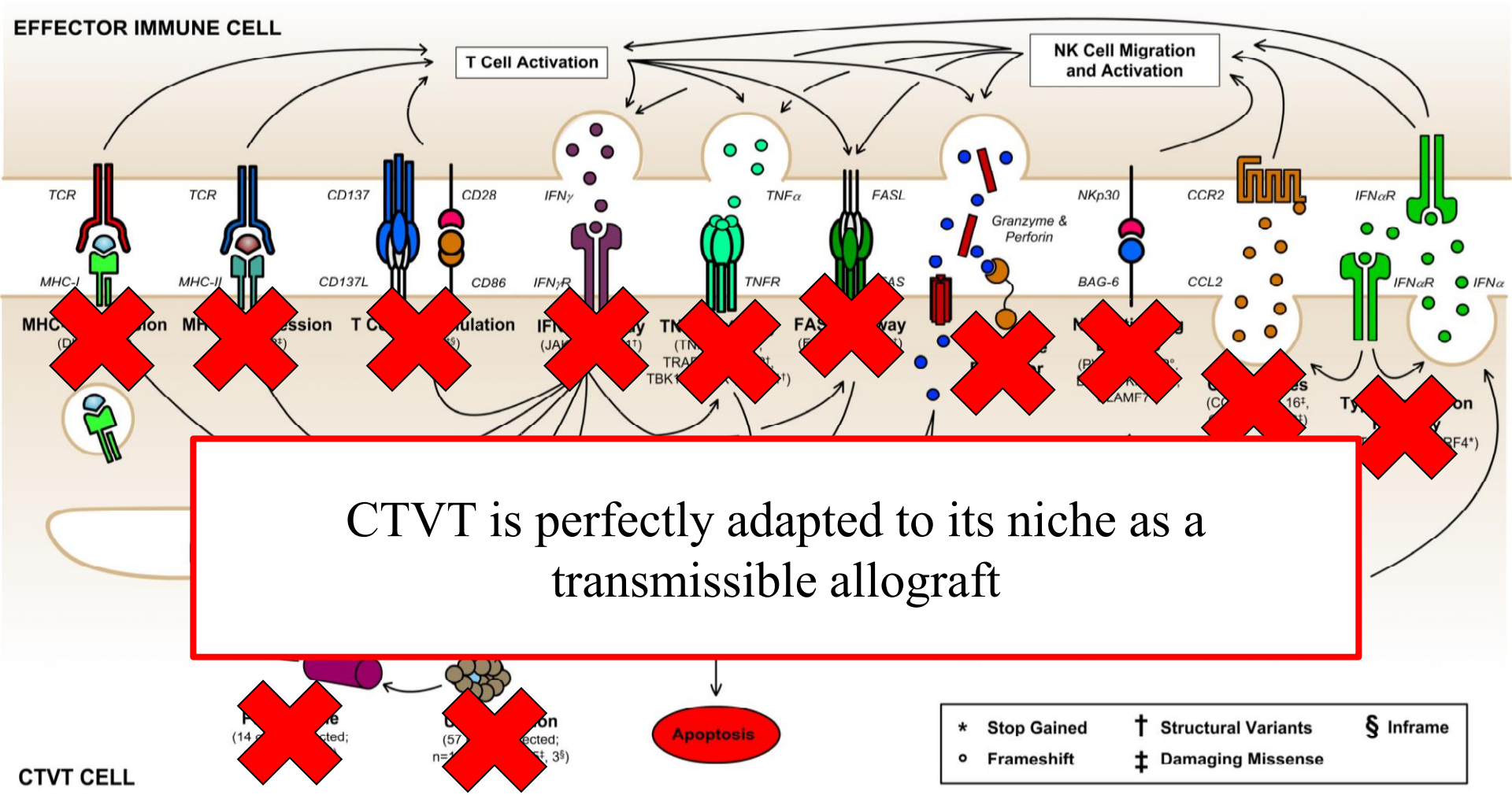
Somatic Mutations

Raw Variants	HQ + Shared	Somatic Candidates
SNV = 8,623,150	SNV = 3,728,916	SNV = 910,376
Indel = 3,683,400	Indel = 1,435,051	Indel = 113,026
SV = 1,531,180	SV = 8,903	SV = 7,338



All steps of antigen presentation are disrupted in CTVT cells, preventing non-self recognition by host T-cells

Somatic Mutations Disrupt All Aspects of Somatic Cell Participation in Immunosurveillance



Unique evolutionary pressures + Thousands of years

=

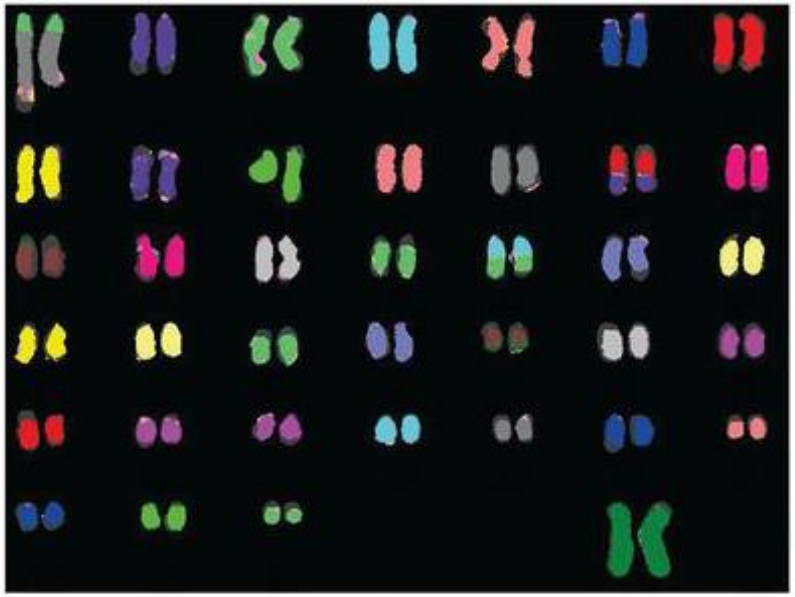
Functionally overlapping somatic mutations enable evasion of host immunosurveillance

Ancient CTVT Mutations Have a Plausible Role in Establishment of Clonal Transmissibility

Immunosurveillance and Cell Cycle Genes with Ancient Somatic Mutations		
CASP3	Translocation in first intron	Executioner caspase – convergence point of intrinsic and extrinsic apoptosis pathways
CDKN2A/B	Deletion	Tumor suppressor – prohibits G1 to S phase transition in the cell cycle
TAP2	Damaging missense, SV	Peptide transporter – essential for loading of MHC class I self-antigen presentation
TP53	Translocation in first intron	Tumor suppressor – cell cycle regulator, apoptosis initiator, DNA repair mediator

- Ancient LOH events: founder inherited allele homozygosity fraction >0.85, somatic mutation homozygosity fraction <0.5
- Ancient somatic mutations: homozygous mutations within those regions

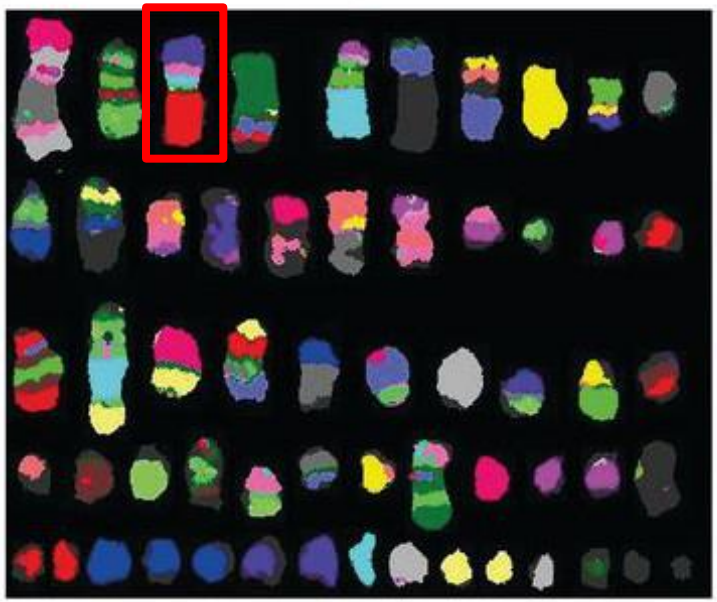
The CTVT Genome is Highly Rearranged

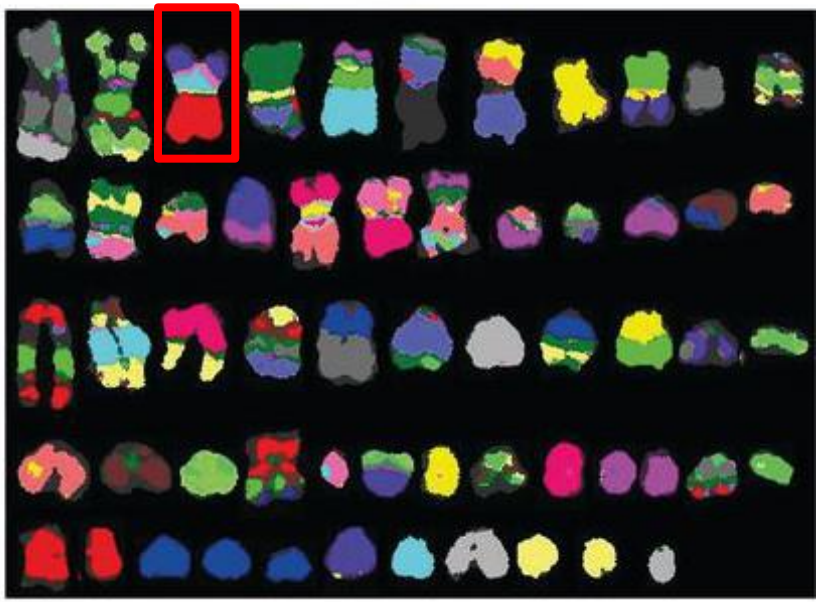


Normal Canine FISH

CTVT FISH (Cape Verde)

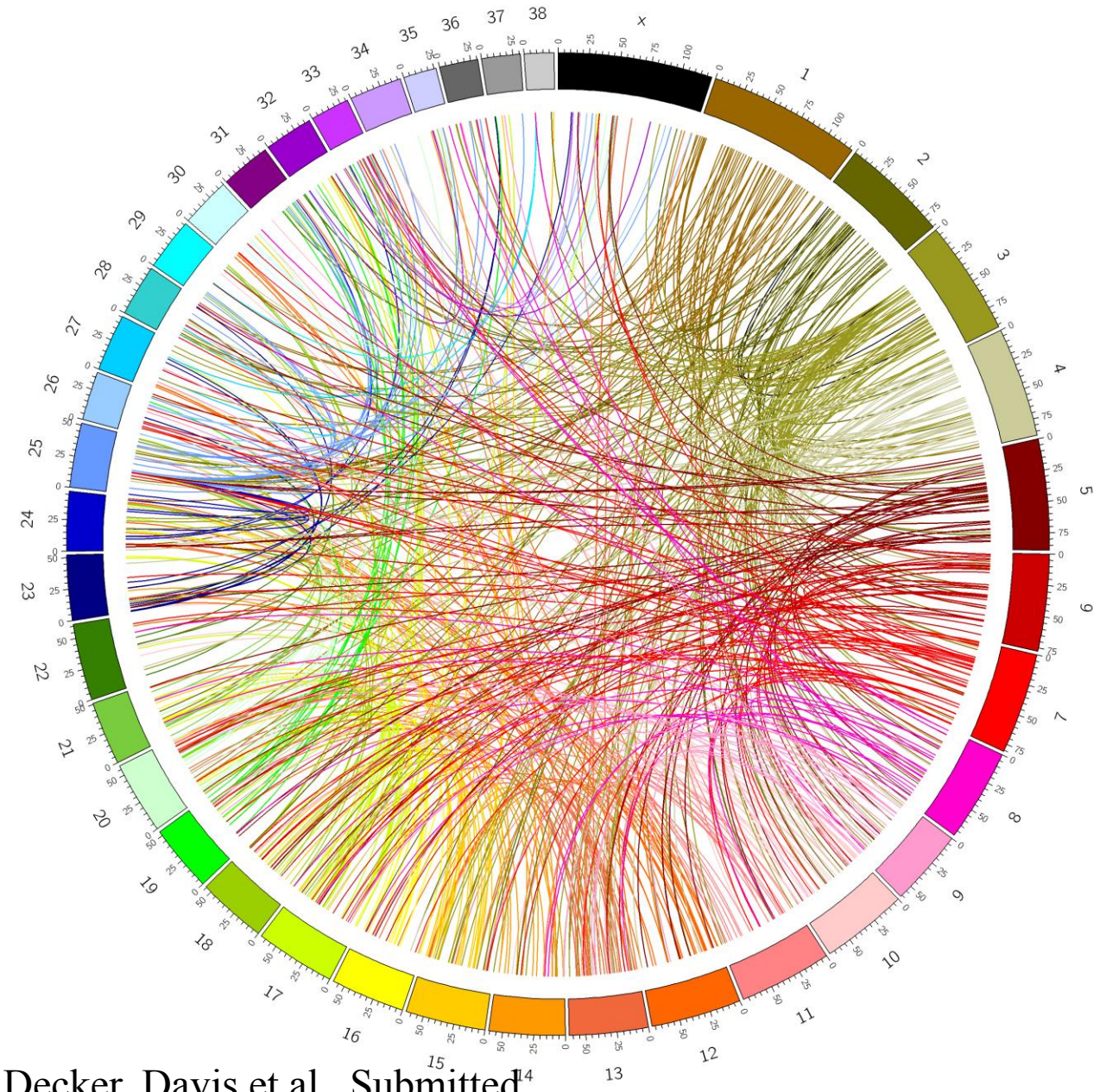
CTVT FISH (Italy)





Murchison, *et al*. *Science*, 2014

Disarrayed Genomic Architecture at Base-Pair Resolution

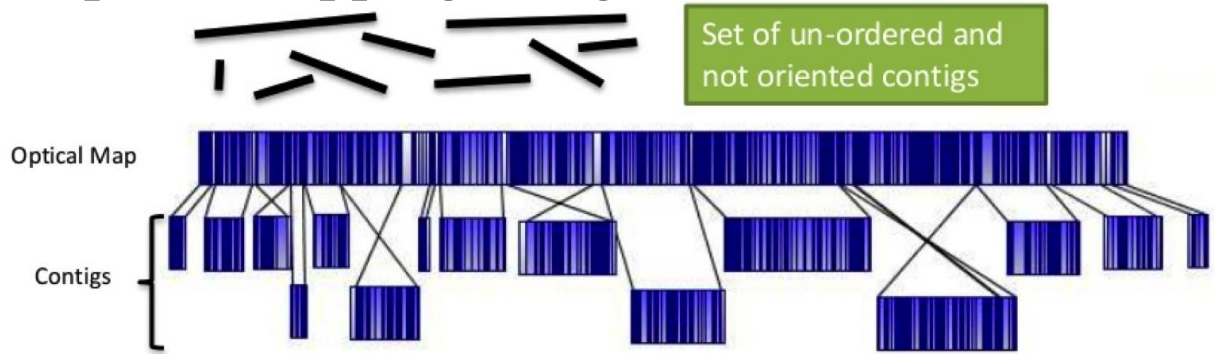


- 7,338 high-quality SVs shared by both tumors
- 2,329 disrupt at least one exon
- 247 potential gene fusions
- Somatic mutations may have contributed to genome instability – *ATM*, *BRCA1*, *BRCA2*, *MRE11A, MLH1*, *PMS1*, *RAD21, TP53*

Decker, Davis et al., Submitted

CTVT-Specific Genome Assembly

- Requisite for annotation and future population and expression studies.
- Obtained fresh frozen samples
  - 150x coverage (Illumina 250bp paired end)
  - PacBio anchoring backbone (10kb-40kb reads)
    - Optical mapping using BioNano IRYS



  - Expression / annotation using total RNA

Conclusions from CTVT

- Our canid variation catalog enables segregation of CTVT founder inherited alleles from somatic drivers of clonal transmissibility
- CTVT is exquisitely adapted to its transmissible allograft niche, with overlapping mutations at every step of somatic cell participation in immunosurveillance
- Identification of some early somatic mutations points to genes that may have contributed to establishing clonal transmissibility
- Understanding CTVT biology may shed light on host-tumor interactions in human cancers
- Sequencing underway for a new CTVT tumor with plans for novel assembly instead of alignment.

Summary

Dogs acquire multiple forms of cancer through natural means as a course of aging, interaction with the environment, and/or inheritance

Analysis of TCC somatic mutations reveal a driver in canine tumors that is identical to a human driver but in an unexpected place

The tumor parasite, CTVT, can provide data regarding how cancers to escape immune detection.

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