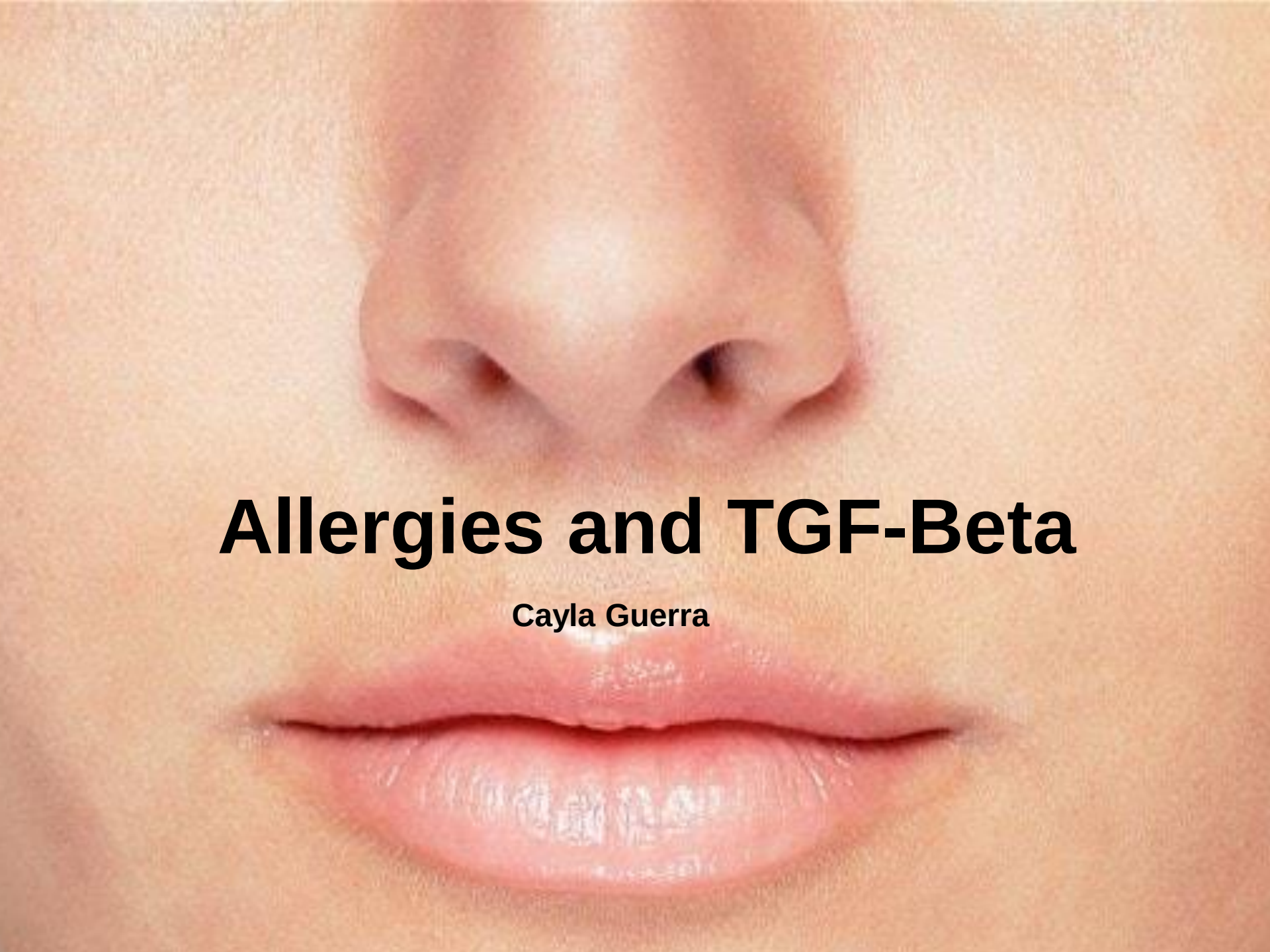
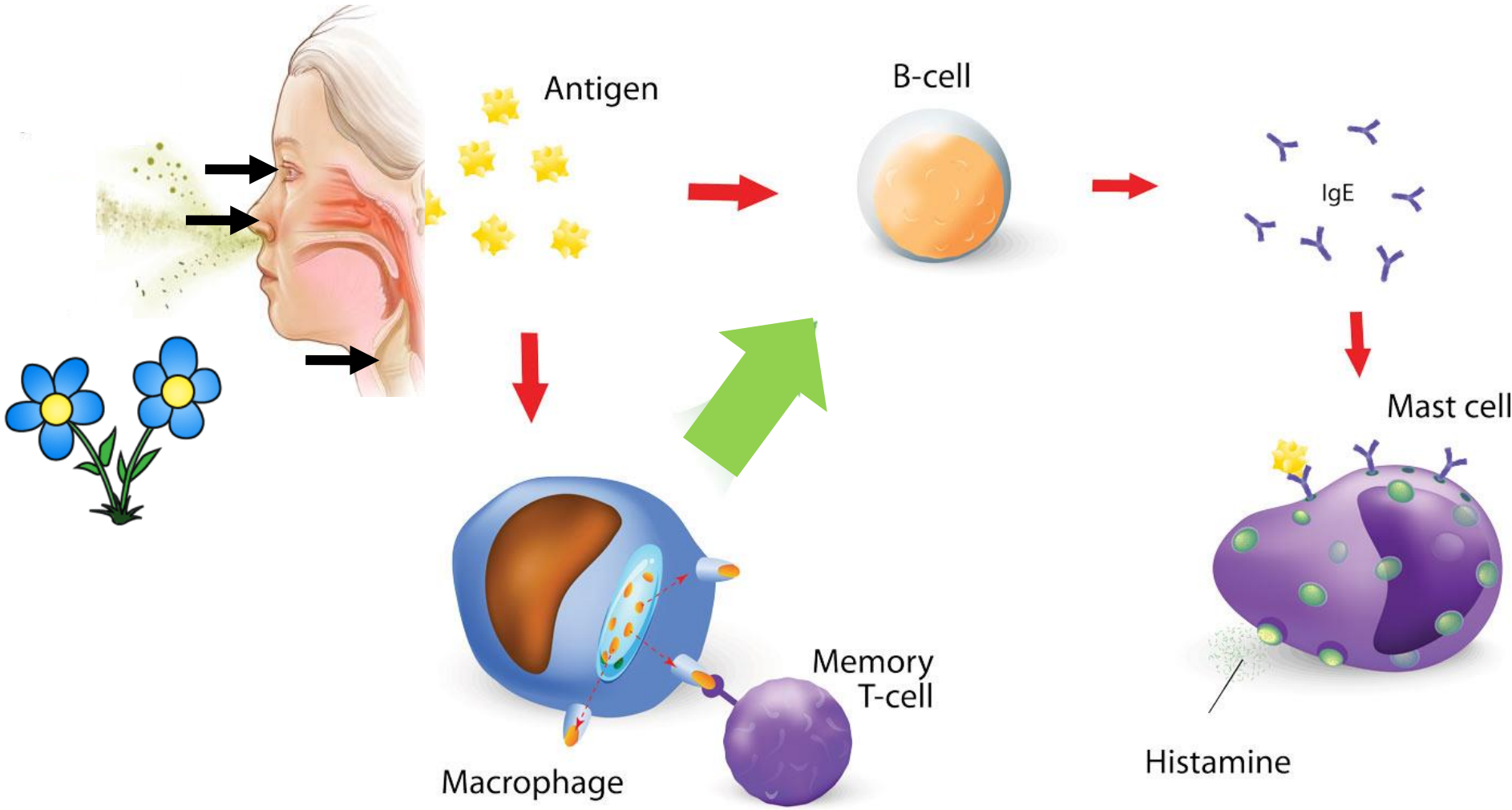


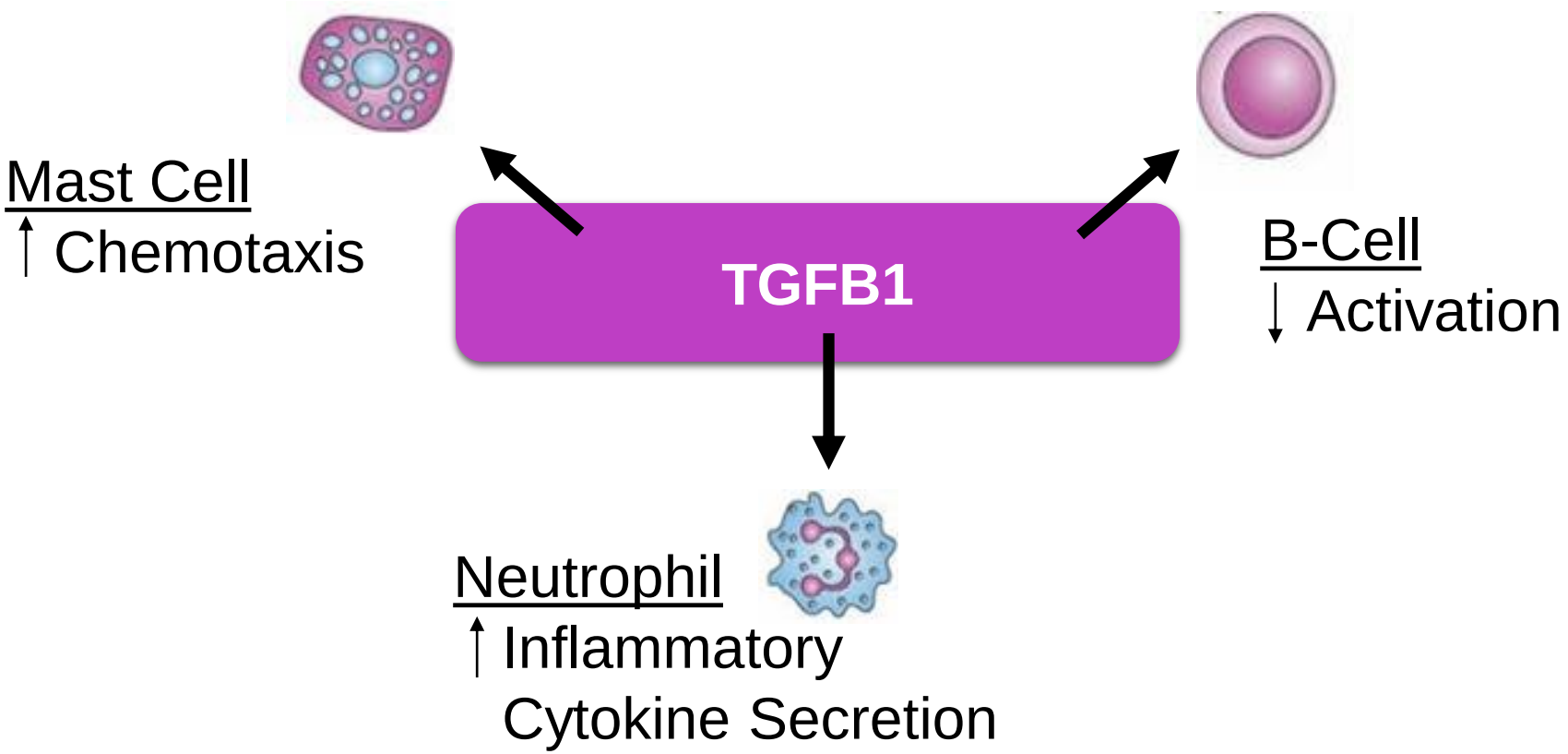
Allergies and TGF-Beta

Cayla Guerra

What is Allergic Rhinitis?



How does TGFB1 function in immunity?

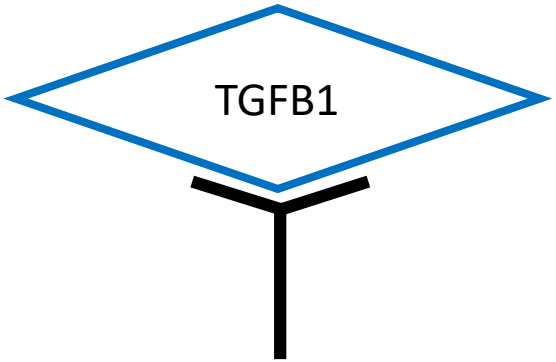


How does TGFB1 function?

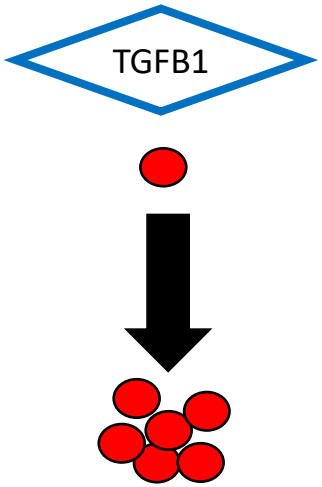
Inactive

Active

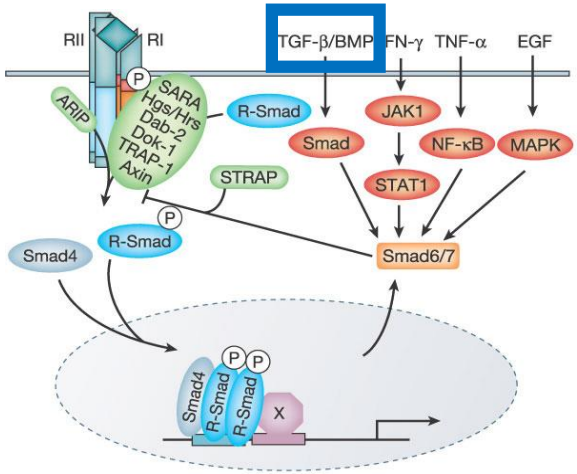
Molecular Function



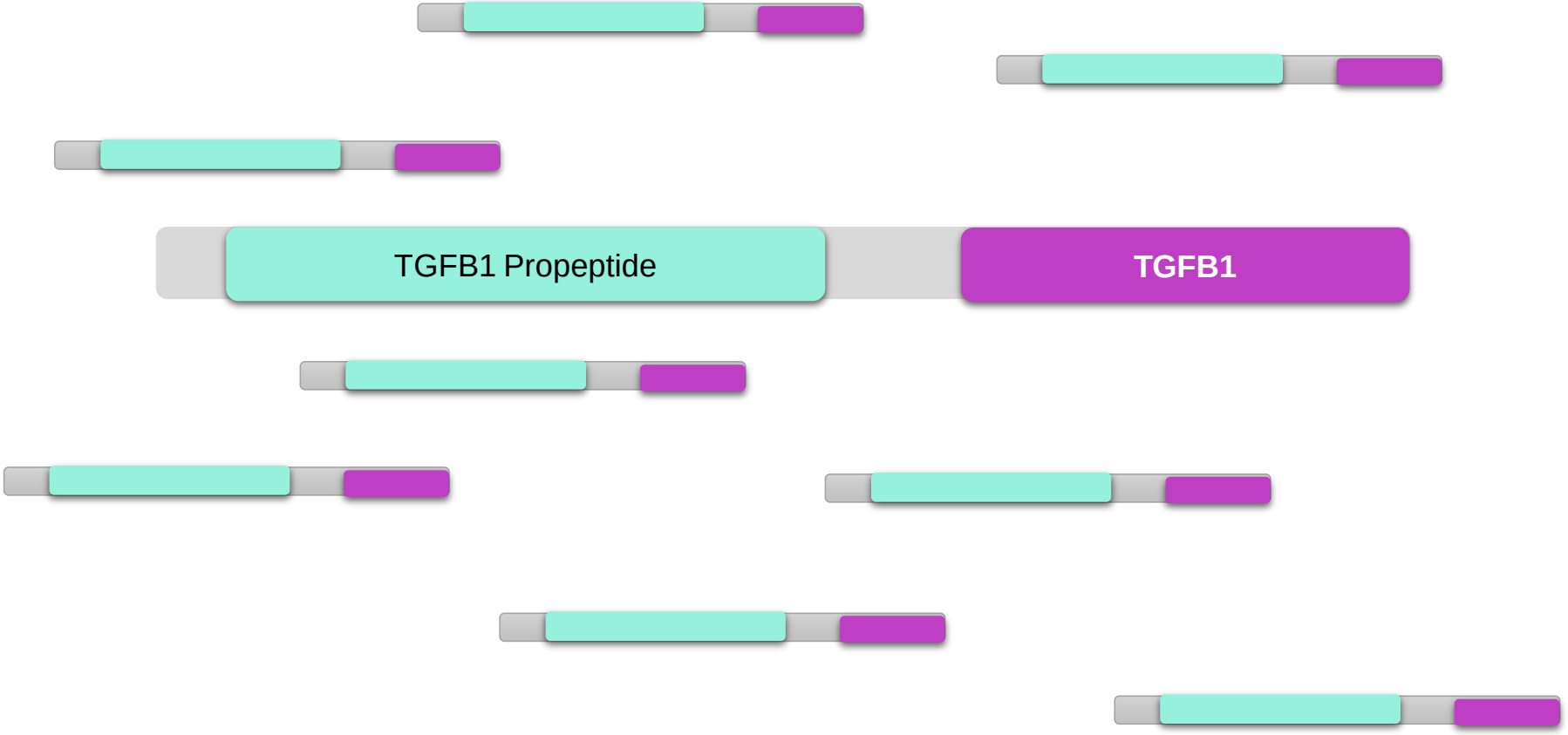
Cellular Component



Biological Process

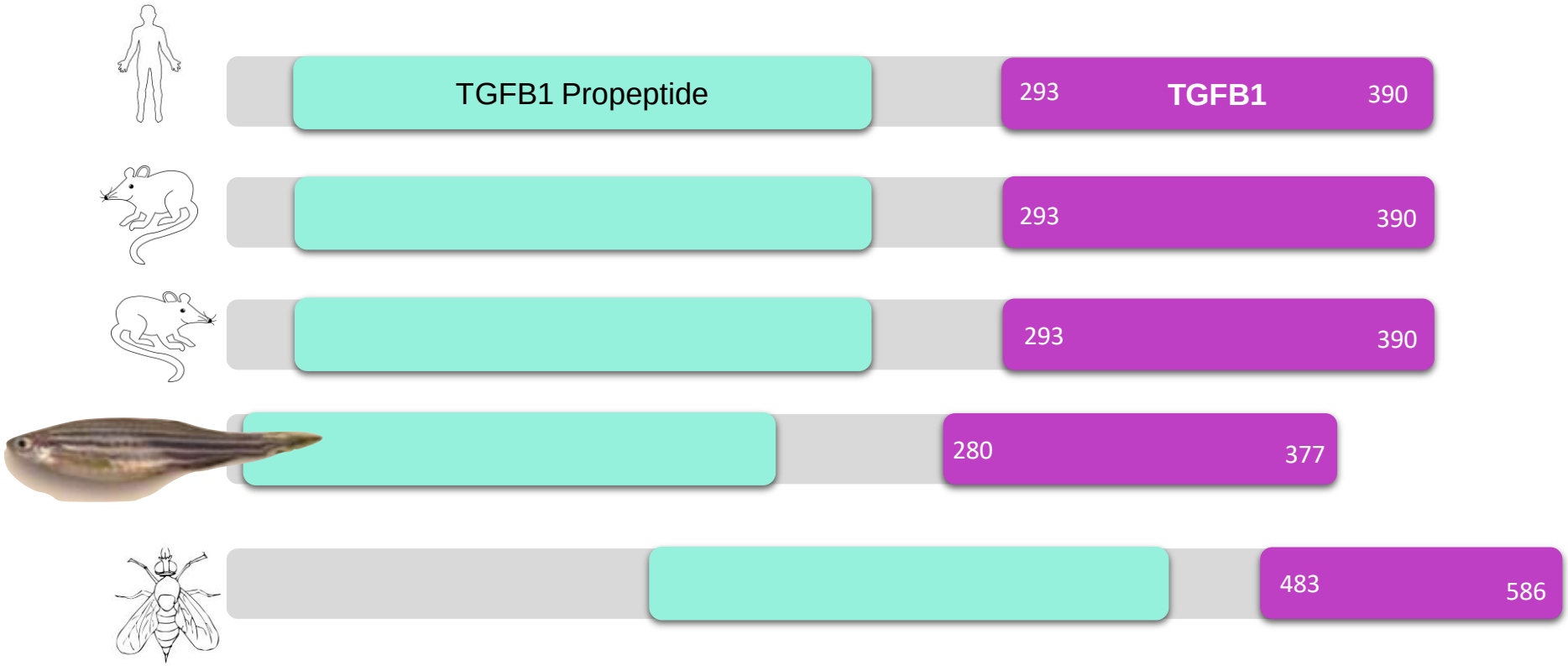


What's wrong with TGF-Beta in Allergic Rhinitis?



It's overexpressed!

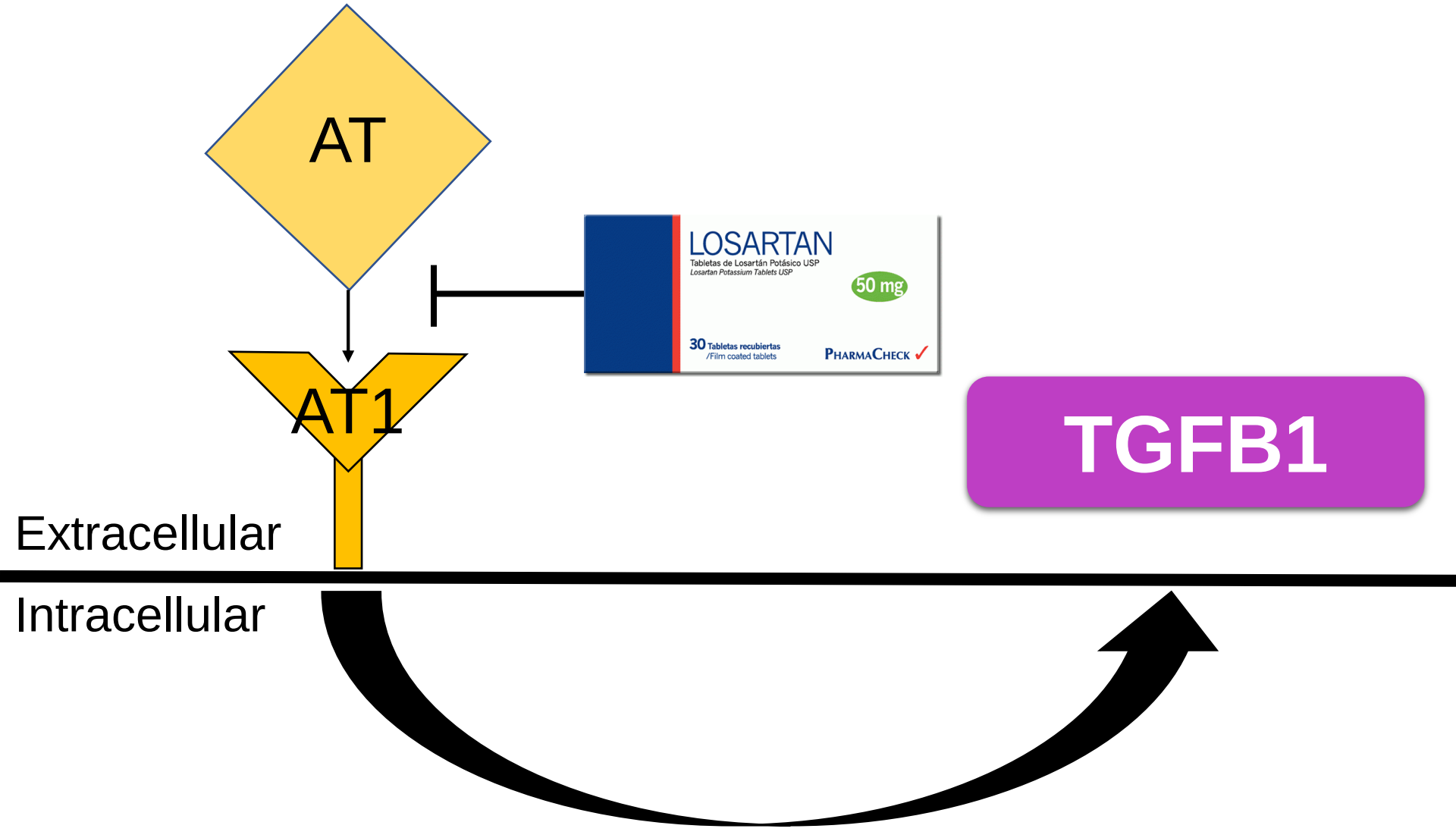
How well is TGF- beta conserved?



How can we treat TGFB1 overexpression?

FDA APPROVED

How does Losartan regulate TGFB1 and blood pressure?



Gap: Does Losartan treat allergies through TGFB1?

Allergic Symptoms

?

TGFB1

Which organism should be used to assay TGFB1 in allergies?

Why are zebra fish useful for TGFB1 allergy assays?

J Appl Toxicol. 2015 Mar;35(3):295-301. doi: 10.1002/jat.3069. Epub 2014 Oct 27.

Tween-80 and impurity induce anaphylactoid reaction in zebrafish.

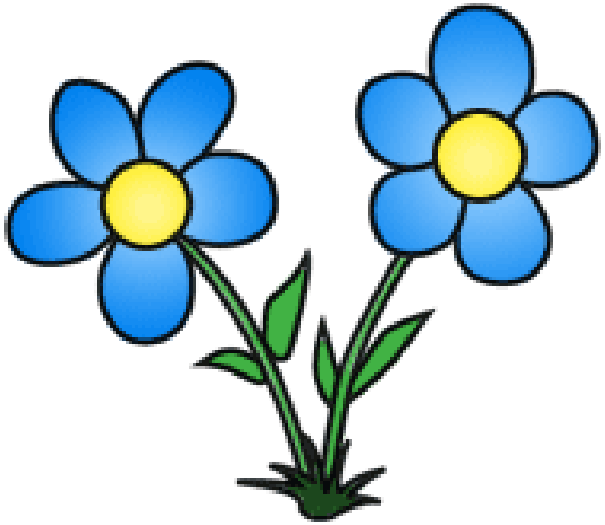
Yang R[1], Lao QC, Yu HP, Zhang Y, Liu HC, Luan L, Sun HM, Li CQ.

A transgenic zebrafish model of neutrophilic inflammation

Stephen A. Renshaw, Catherine A. Loynes, Daniel M.I. Trushell, Stone Elworthy, Philip W. Ingham and Moira K.B. Whyte

Blood 2006 108:3976-3978; doi: https://doi.org/10.1182/blood-2006-05-024075

How can we induce an allergic response in zebra fish?



=

Polysorbate 80 (Tween-80) & hydrogen peroxide in trace amounts dropped into tank water

Primary Goal

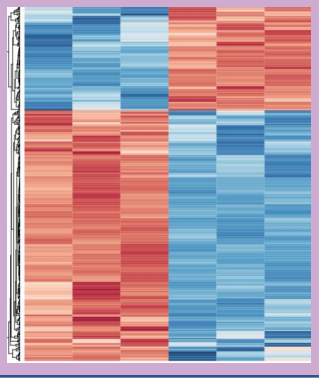
To determine if Losartan can decrease the inflammatory symptoms associated with allergic rhinitis

Determine conserved amino acids

DrosophilaDawdle	KSLELVPCCTAKQ
ZebraFishTGFB1a	PGASAQPCCVPAI
MouseTGFB1	PGASASPCCVPQA
RatTGFB1	PGASASPCCVPQA
HorseTGFB1	PGASAAPCCVPQV
DogTGFB1	PGASAAPCCVPQA
HumanTGFB1	PGASAAPCCVPQA
ChimpanzeeTGFB1	PGASAAPCCVPQA
CowTGFB1	PGASAAPCCVPQA

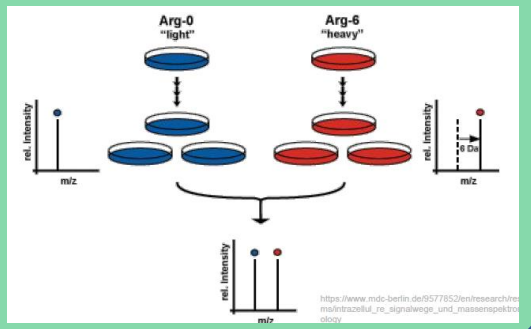
Aim 1

Gene expression patterns with and without Losartan



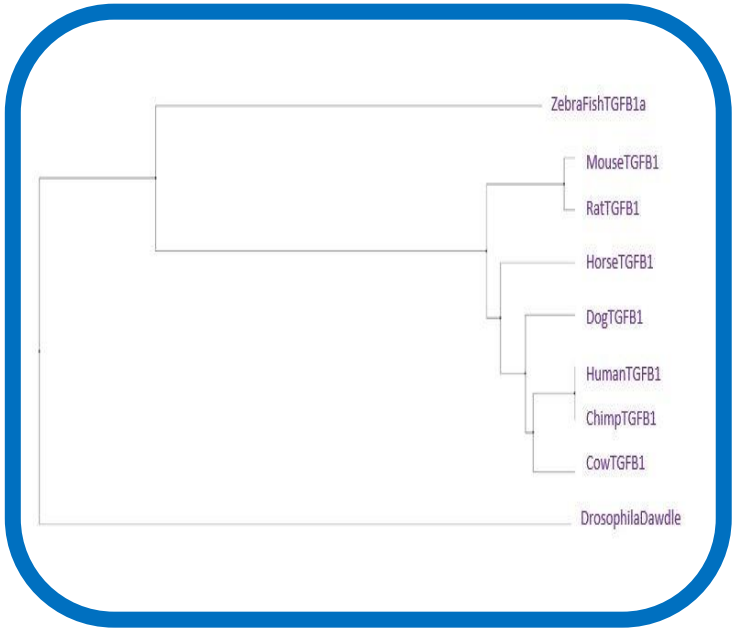
Aim 2

Quantity of proteins with and without Losartan



Aim 3

Aim 1: Determine conserved amino acids in TGFB1 that are important for inflammation



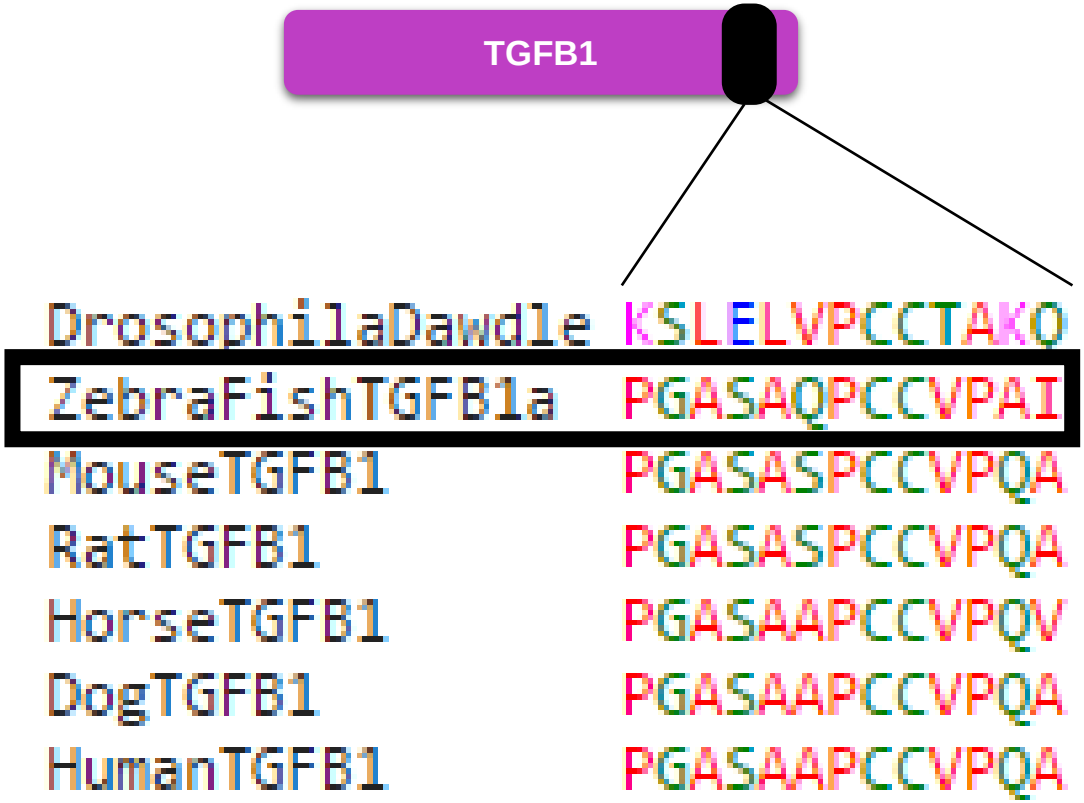

```
TECCREHLYISFRD-I
ETCCMRKLYIDFRKDL
KNCCVRQLYIDFRKDL
KNCCVRQLYIDFRKDL
KNCCVRQLYIDFRKDL
KNCCVRQLYIDFRKDL
KNCCVRQLYIDFRKDL
KNCCVRQLYIDFRKDL
KNCCVRQLYIDFRKDL
  ** .:***.**. :

GANKSLELVPCCTAKQ
HHNPGASAQPCCVPAI
QHNPGASASPCCVPQA
QHNPGASASPCCVPQA
QHNPGASAAPCCVPQV
QHNPGASAAPCCVPQA
QHNPGASAAPCCVPQA
QHNPGASAAPCCVPQA
QHNPGASAAPCCVPQA
```

DrosophilaDawdle
ZebraFishTGFB1a
MouseTGFB1
RatTGFB1
HorseTGFB1
DogTGFB1
HumanTGFB1

Organisms with similar immune systems have conserved regions

Clustal Omega → Identify regions → CRISPR


TGFB1
DrosophilaDawdle KSLELVPCCTAKQ
ZebraFishTGFB1a PGASAQPCCVPAI
MouseTGFB1 PGASASPCCVPQA
RatTGFB1 PGASASPCCVPQA
HorseTGFB1 PGASAAPCCVPQV
DogTGFB1 PGASAAPCCVPQA
HumanTGFB1 PGASAAPCCVPQA

Possible Immunologic motifs


```
DrosophilaDawdle   KSLELVPCCTAKQ
ZebraFishTGFB1a    PGASAQPCCVPAI
MouseTGFB1         PGASASPCCVPQA
RatTGFB1           PGASASPCCVPQA
HorseTGFB1         PGASAAPCCVPQV
DogTGFB1           PGASAAPCCVPQA
HumanTGFB1         PGASAAPCCVPQA

DrosophilaDawdle   HHSSIMKILSTSGAN
ZebraFishTGFB1a    QILA---LY--KHHN
MouseTGFB1         KVLA---LY--NQHN
RatTGFB1           KVLA---LY--NQHN
HorseTGFB1         KVLA---LY--NQHN
DogTGFB1           KVLA---LY--NQHN
HumanTGFB1         KVLA---LY--NQHN
```

Less probable immunologic motifs


```
DrosophilaDawdle   SGHELSHLIQI
ZebraFishTGFB1a    QGSEDEETLEL
MouseTGFB1         NQGDGIQGFRF
RatTGFB1           NQGDGIQGFRF
HorseTGFB1         SQGGAMEGLRL
DogTGFB1           SHGGEVEGFRL
HumanTGFB1         SRGGEIEGFRL

DrosophilaDawdle   NVQDEWMKI
ZebraFishTGFB1a    DLSNRWLSF
MouseTGFB1         TDTPEWLSF
RatTGFB1           TDTPEWLSF
HorseTGFB1         SDSPEWLSF
DogTGFB1           SDTPEWLSF
HumanTGFB1         SDSPEWLSF
```

Can we induce inflammation in these knockouts?

PGASAQPCCVPAI

Knockout 1

QILA---LY--KHHN

Knockout 2

QGSEDEETLEL

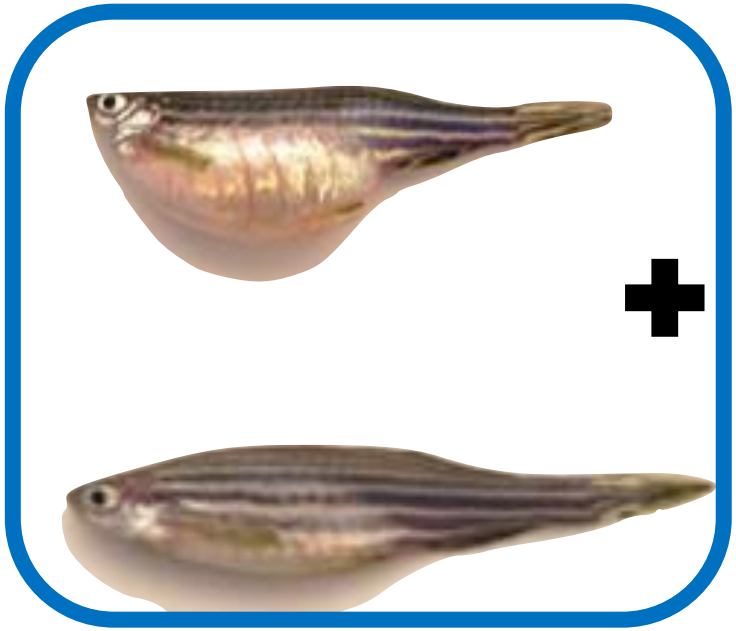
Knockout 3

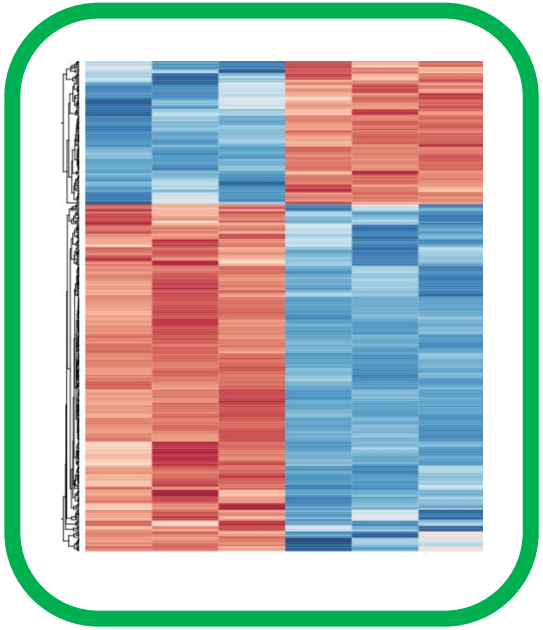
DLSNRWLSF

Knockout 4

Clustal Omega | Identify regions | CRISPR

Aim 2: Determine changes in expression of TGFB1 and ontology categories with or without treatment



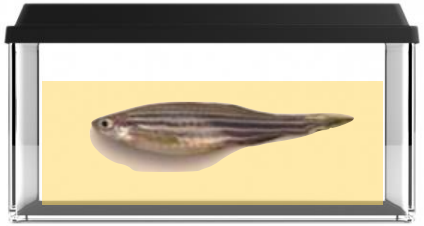


Immunologic and blood pressure maintenance genes will be downregulated after treatment

Allergen → Losartan → RNA-Seq

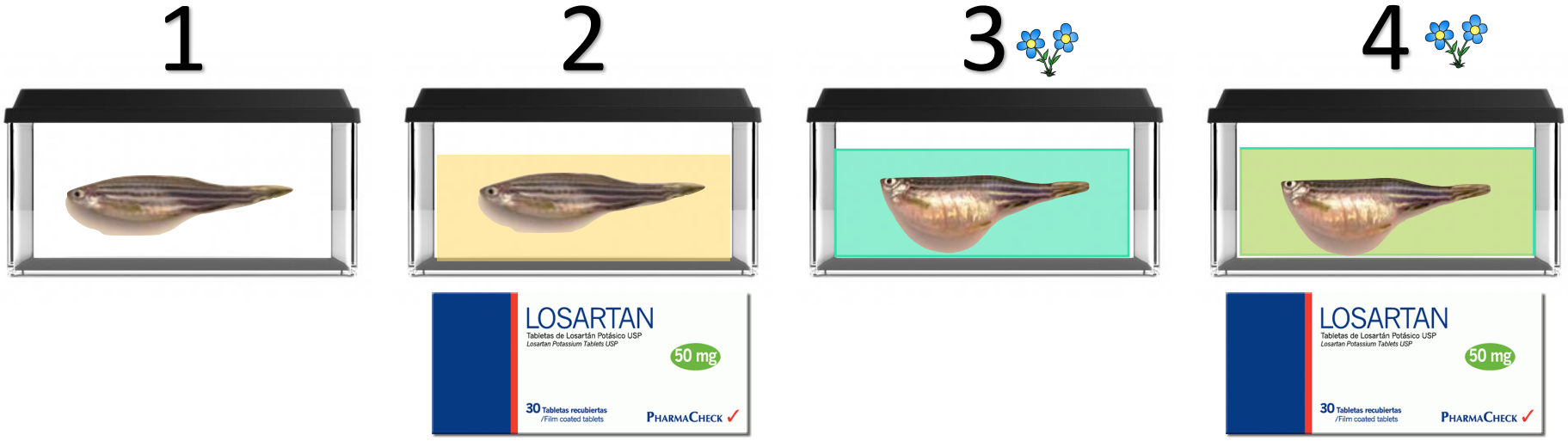
1

2



Wt

Wt+Losartan



Wt

Wt+Losartan

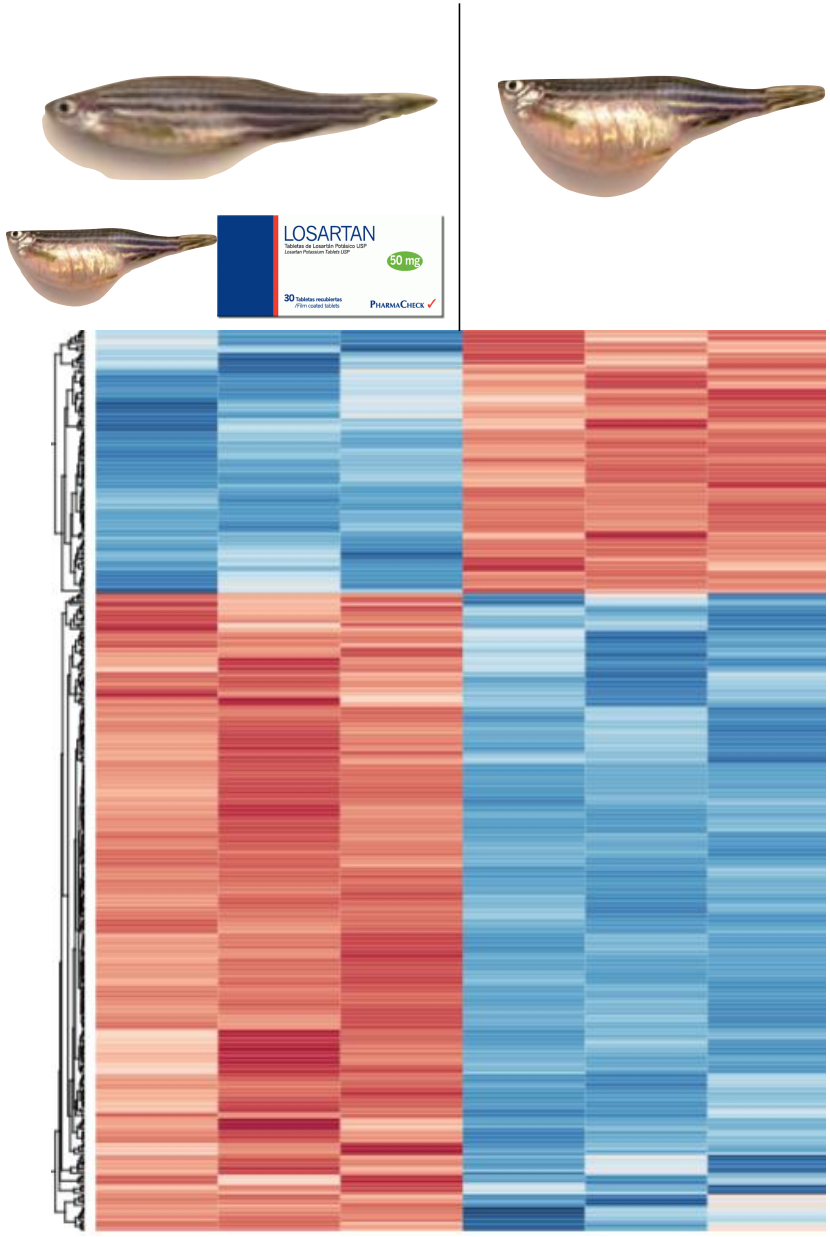
Allergic

Allergic +Losartan

RNA Sequencing

Gene Ontology

Allergen | Losartan | RNA-Seq

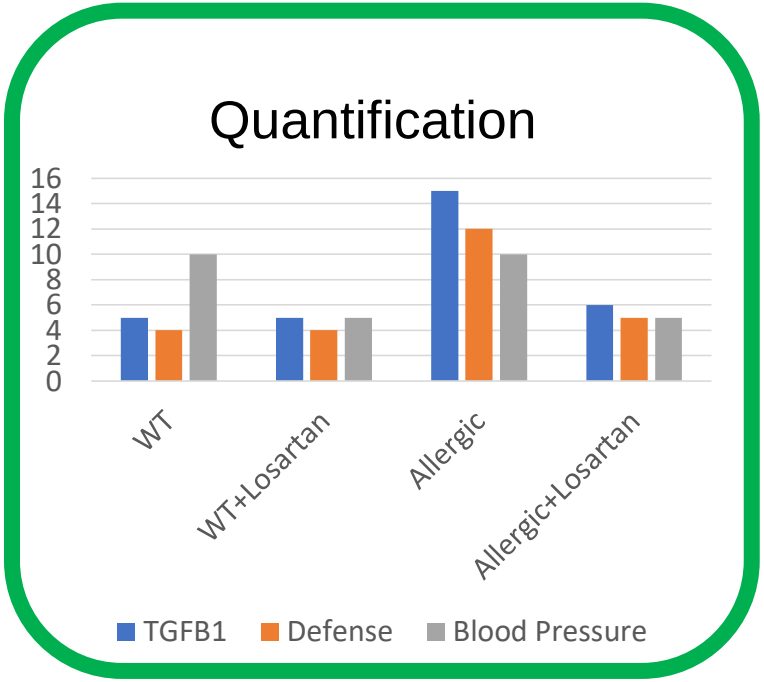


At least 2x difference

Immunologic genes

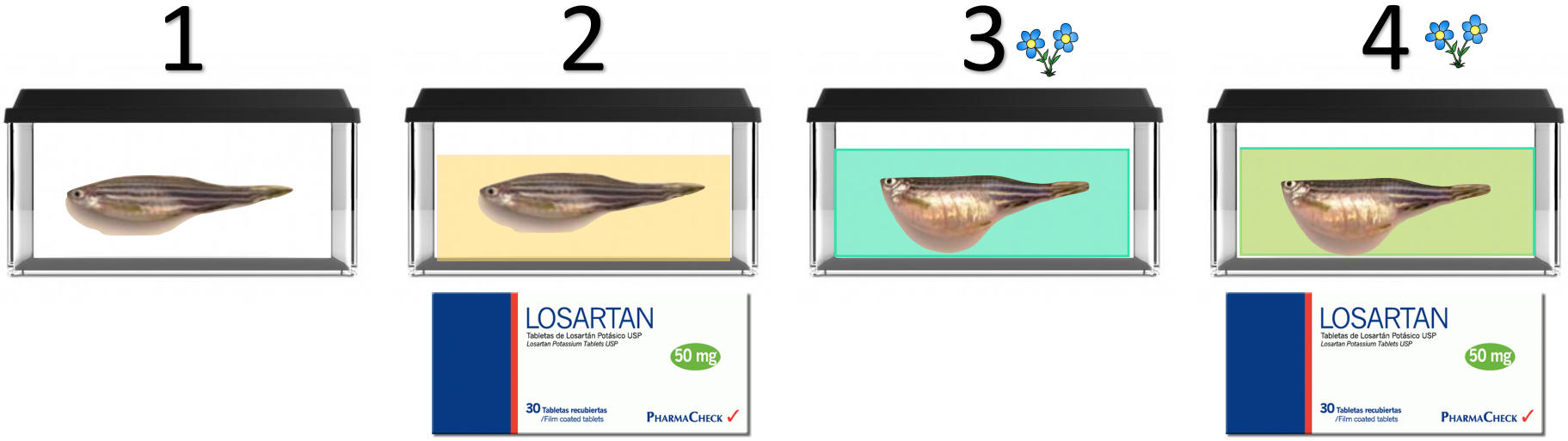
Blood pressure maintenance genes

Aim 3: Quantify protein levels of TGFB1 with and without treatment with Losartan



Immunologic proteins will increase in allergic fish. Losartan treated fish will have diminished blood pressure maintenance proteins

Treat | SILAC | Mass Spec



Wt

Wt+Losartan

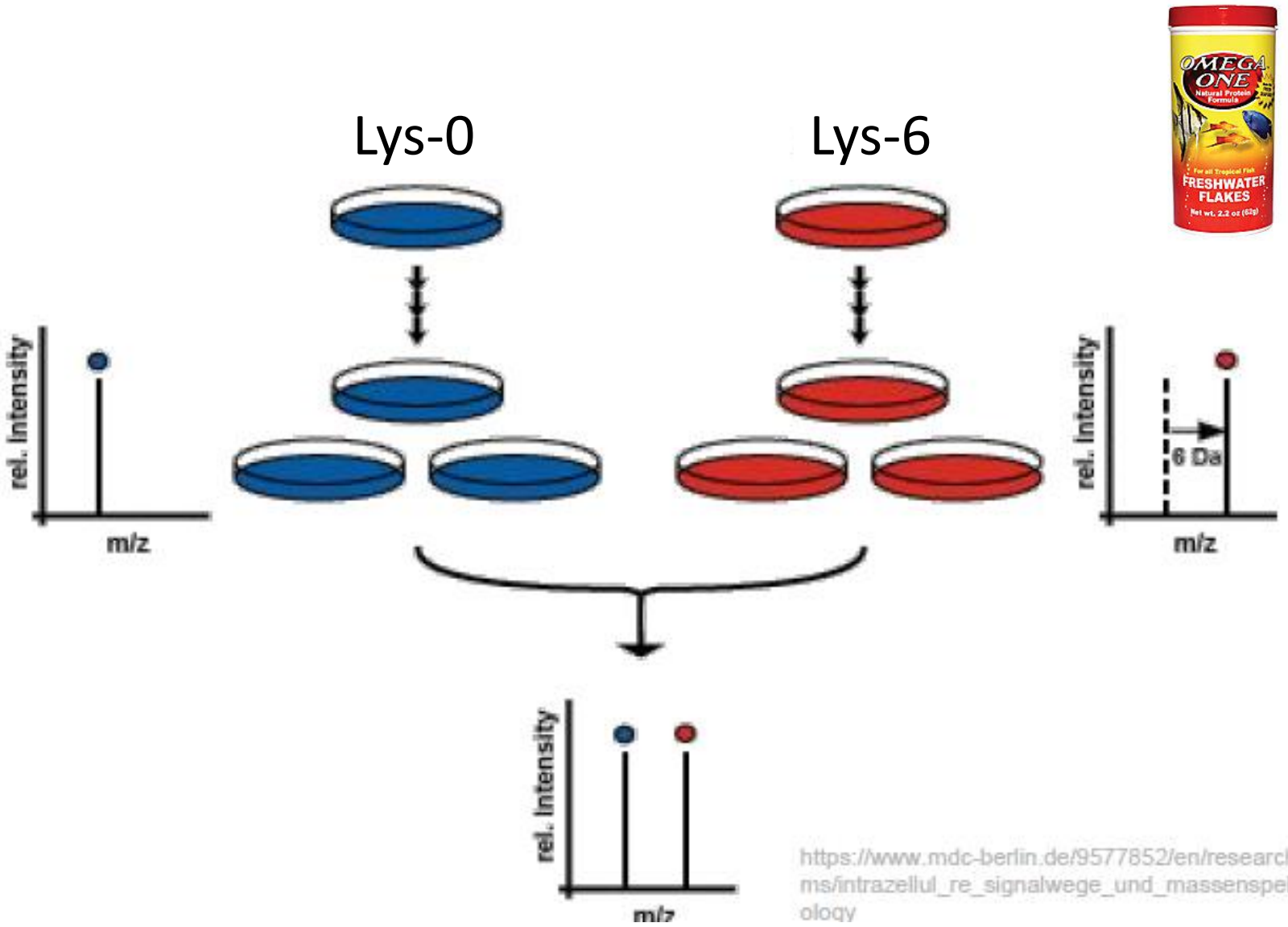
Allergic

Allergic +Losartan

SILAC

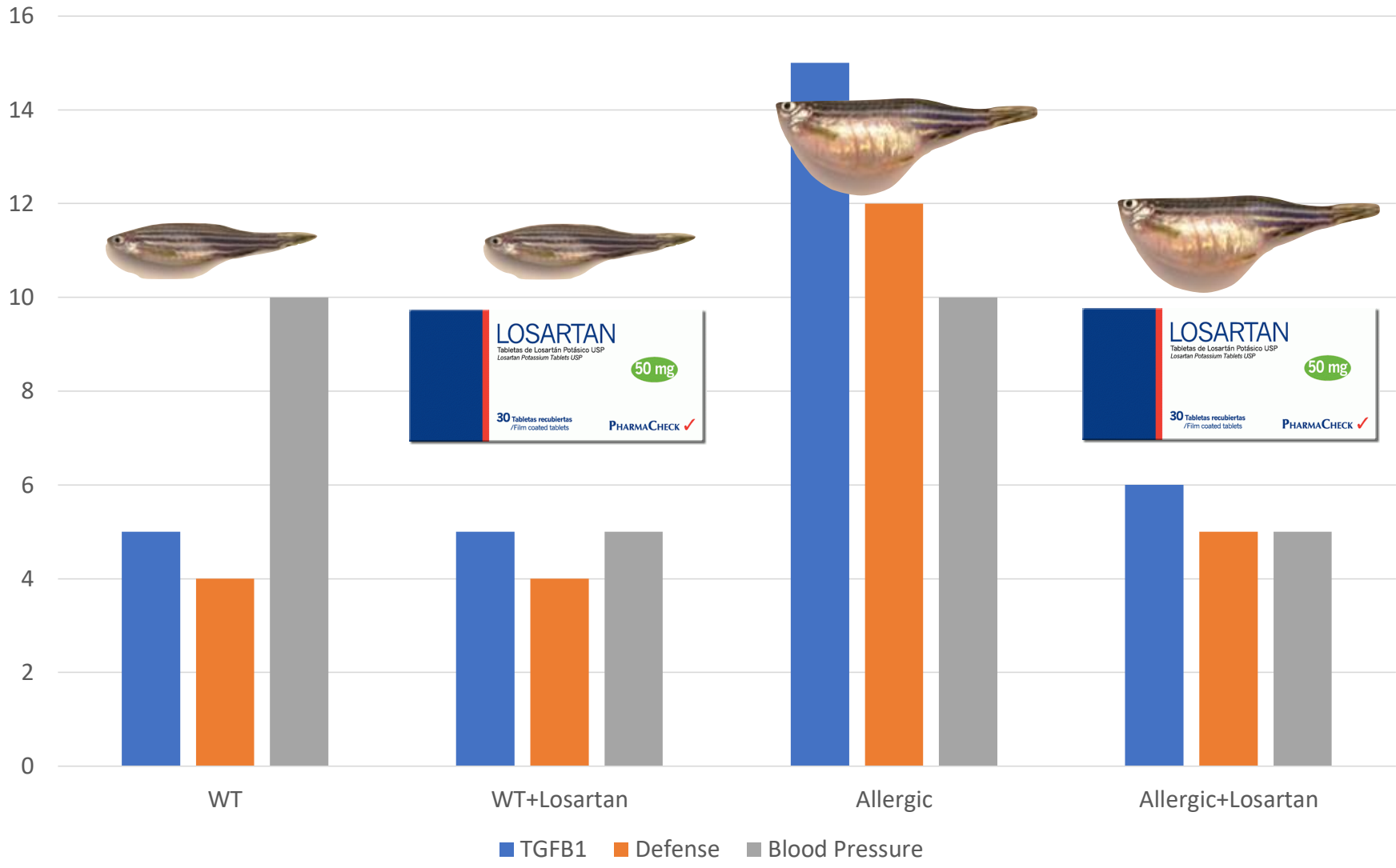
Mass Spectrometry

Allergen and Drug | SILAC | Mass Spec



https://www.mdc-berlin.de/9577852/en/research/res
ms/intrazellul_re_signalwege_und_massenspektro
ology

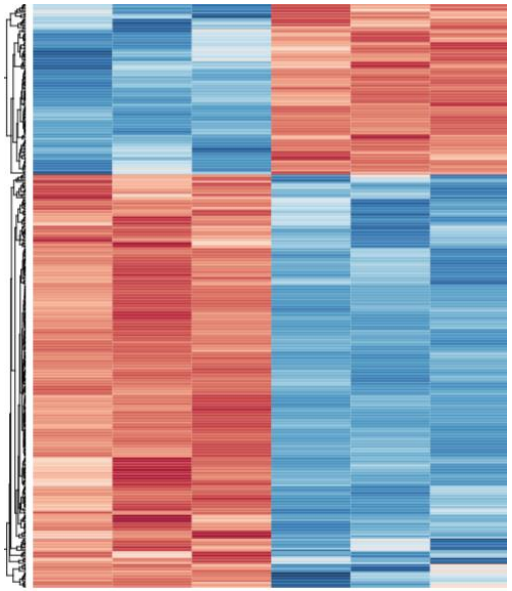
Quantification

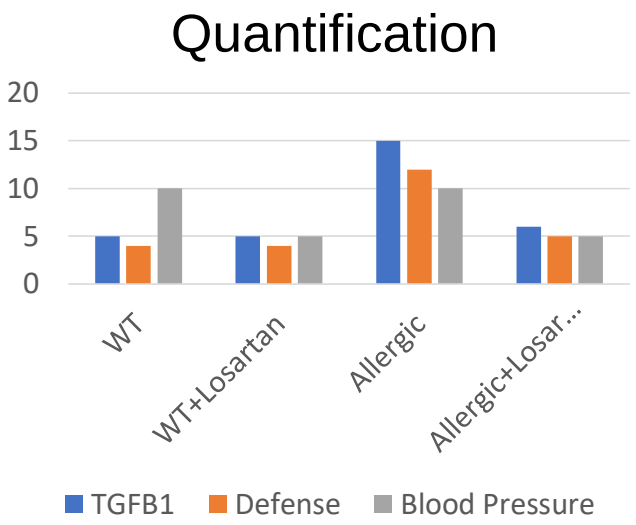


Allergen and Drug | SILAC | Mass Spec

Summary

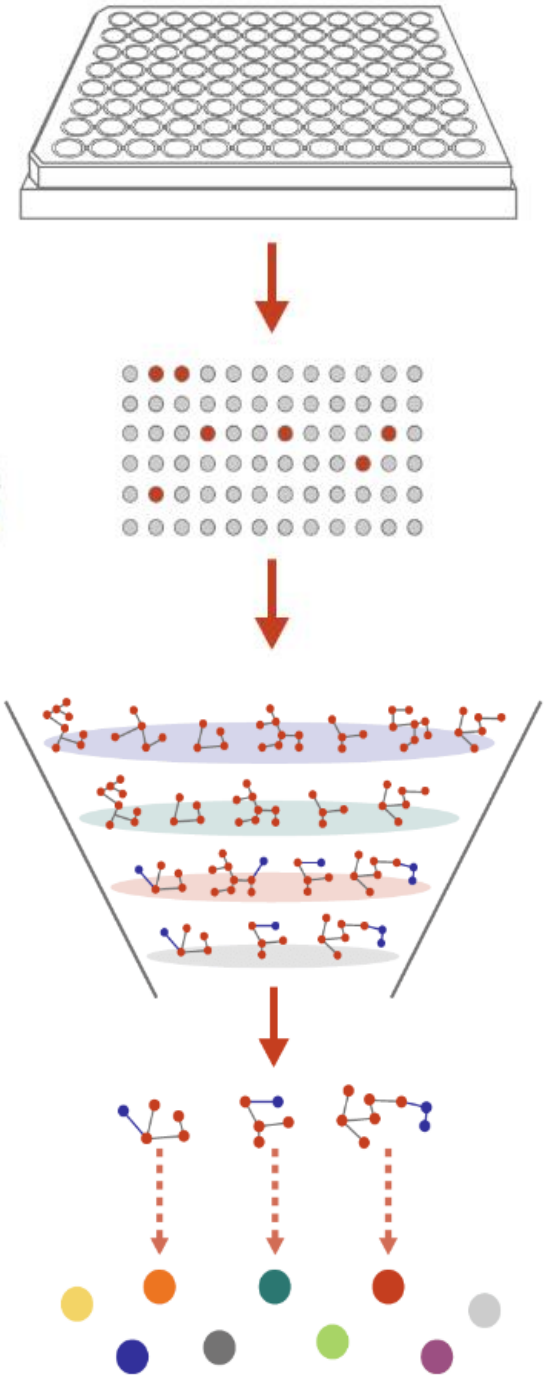
```
DrosophilaDawdle KSLELVPCCTAKQ
ZebraFishTGFB1a  PGASAQPCCVPAI
MouseTGFB1       PGASASPCCVPQA
RatTGFB1         PGASASPCCVPQA
HorseTGFB1       PGASAAPCCVPQV
DogTGFB1         PGASAAPCCVPQA
HumanTGFB1       PGASAAPCCVPQA
```



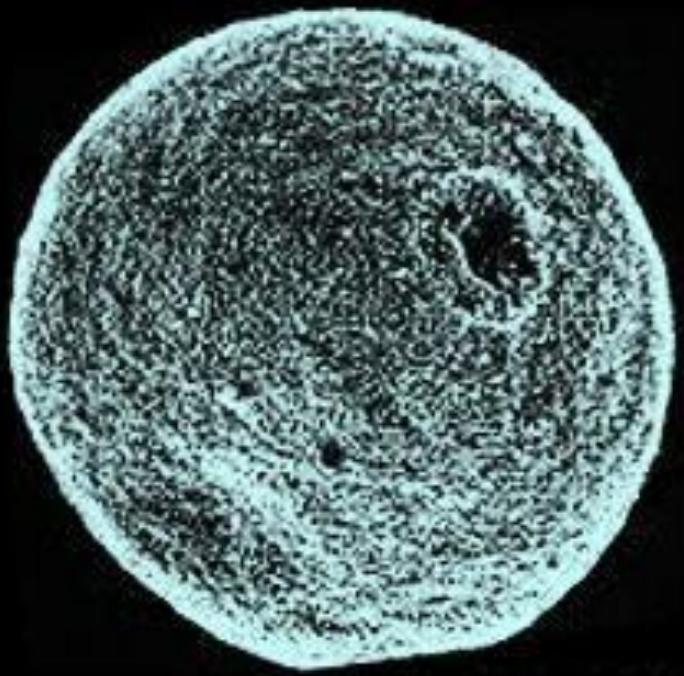


Allergic Symptoms

Future directions



POLLEN



DEATH STAR

ANY QUESTIONS?

References

[1] Wan, Y. Y., & Flavell, R. A. 2007. "Yin-Yang" functions of TGF-β and Tregs in immune regulation. *Immunological Reviews*, *220*, 199–213. http://doi.org/10.1111/j.1600-065X.2007.00565.x

[2] Debock I., Flamand V. 2014. Unbalanced Neonatal CD4+ T-cell Immunity. *Frontiers in Immunology, 393.* 1664-3224. https://doi.org/10.3389/fimmu.2014.00393

[3] Ripley, E., & Hirsch, A. (2010). Fifteen years of losartan: what have we learned about losartan that can benefit chronic kidney disease patients? *International Journal of Nephrology and Renovascular Disease*, *3*, 93–98.

[4] Renshaw *et* al. (2006). A transgenic zebrafish model of neutrophilic inflammation. *American Society of Hematology.* 108:3976-3978. https://doi.org/10.1182/blood-2006-05-024075

[5] Yang *et* al. (2014). Tween-80 and impurity induce anaphylactoid reaction in zebra fish. *Journal of applied Toxicology*. 35,3:295-301. 10.1002/jat.3069

[6] Konzer *et* al. (2013). Stable Isotope Labeling in Zebrafish Allows in Vivo Monitoring of Cardiac Morphogenesis. *Mol Cell Proteomics*. 12(6): 1502-1512. 10.1074/mcp.M111.015594