Wnt Signaling in Metastatic Cancers

Taran Gujral, Ph.D.
Department of Systems Biology
Harvard Medical School

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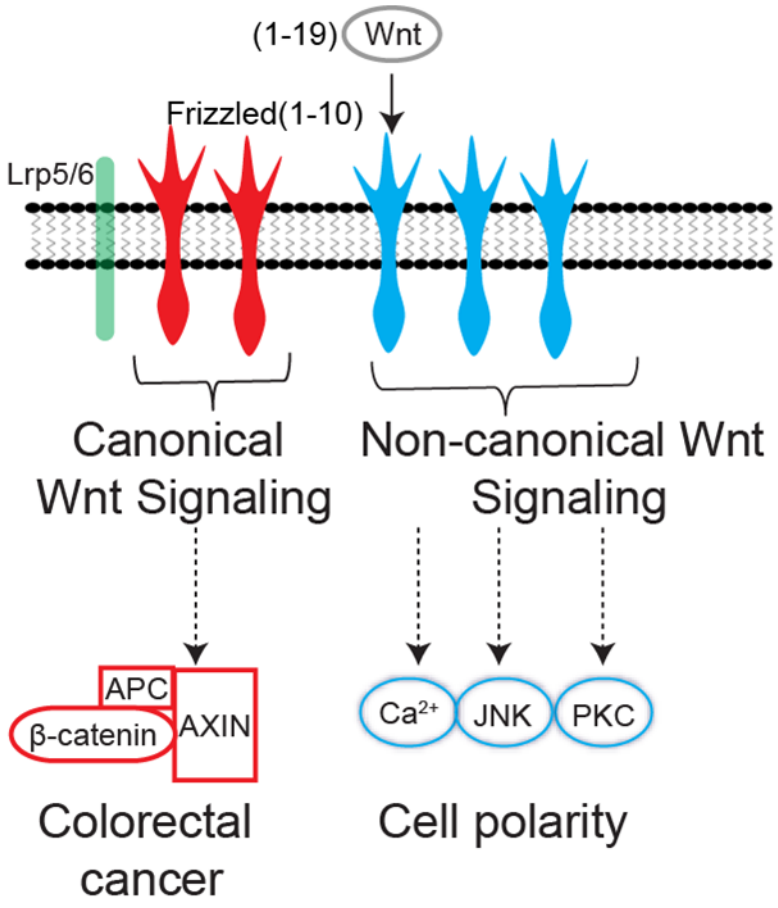
Wnt signaling in cancer

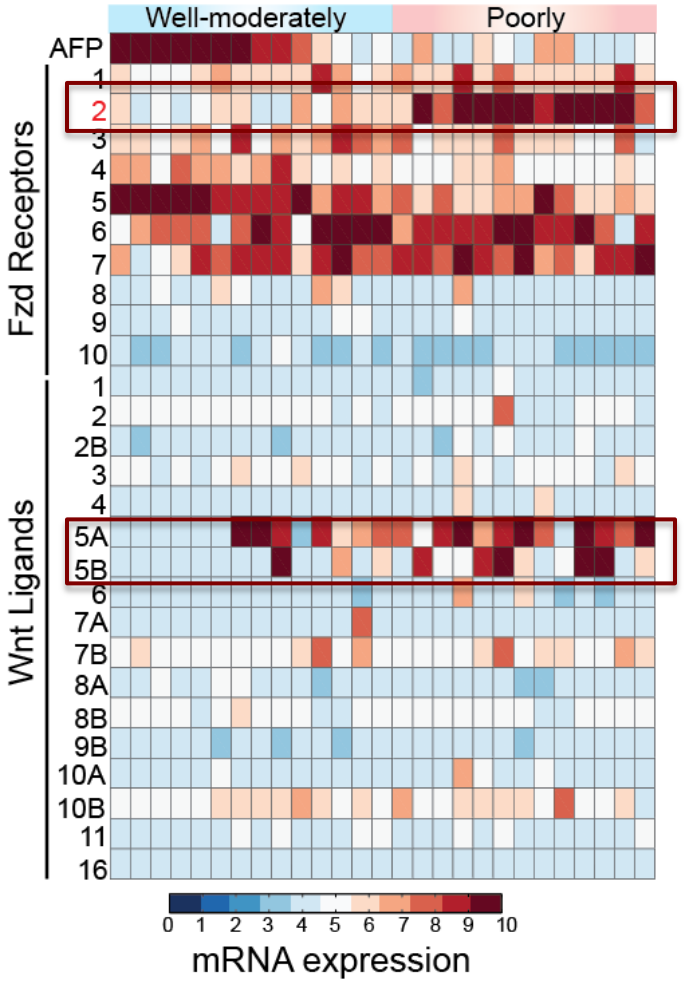


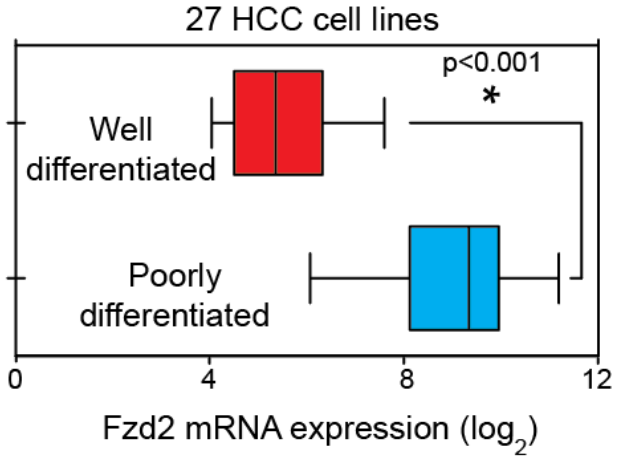
Oncomed: OMP-18R5
Novartis: XAV939
Novartis: LGK974
Prism/Eisai:PRI-724

- Mutations in components of the Wnt signaling pathway have been well-documented in cancer, but are largely confined to colorectal cancers
- To date, drug development has focused largely on the canonical signaling pathway, which centers on β-catenin

Fzd2 and Wnt5a/b are highly expressed in late-stage hepatocellular carcinoma (HCC) cell lines

mRNA expression of Fzd receptors and Wnt ligands in 27 HCC cell lines



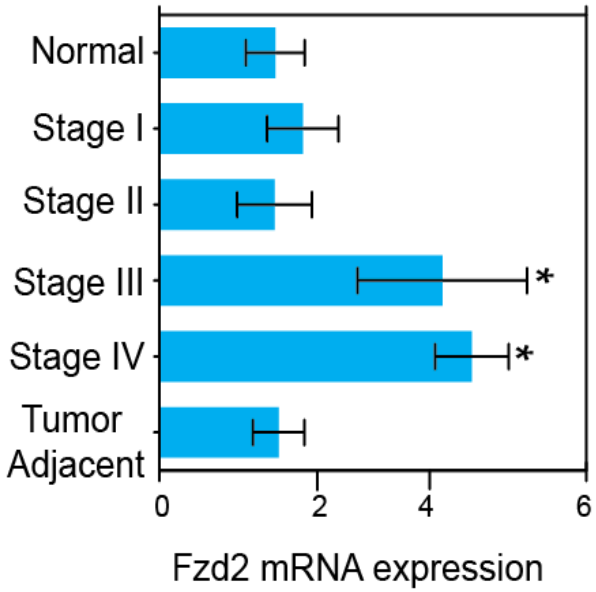


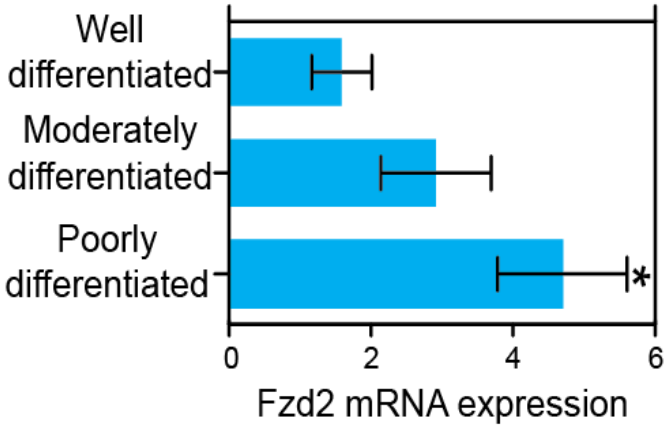
Fzd2 and **Wnt5a/b** are highly expressed in **poorly differentiated** HCC cell lines

Fzd2 is highly expressed in late-stage HCC

Fzd2 mRNA expression in 48 primary HCC tumors

A.U. normalized to normal or well differentiated samples

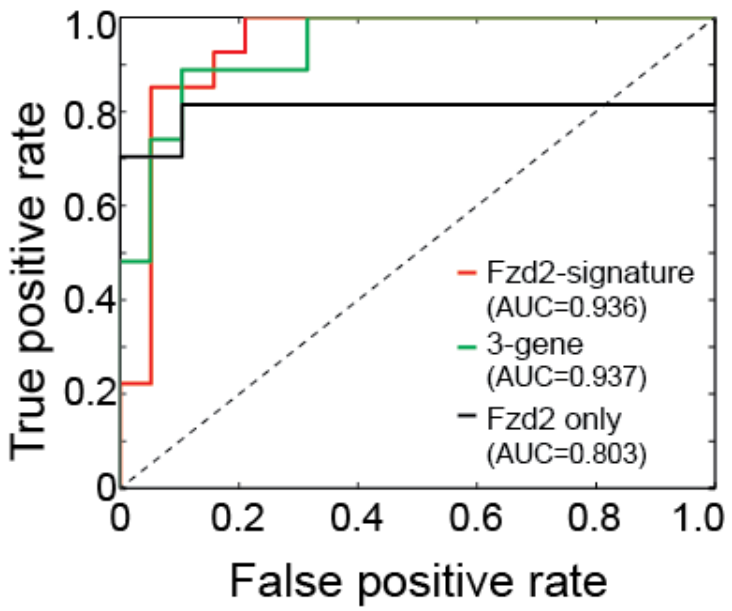




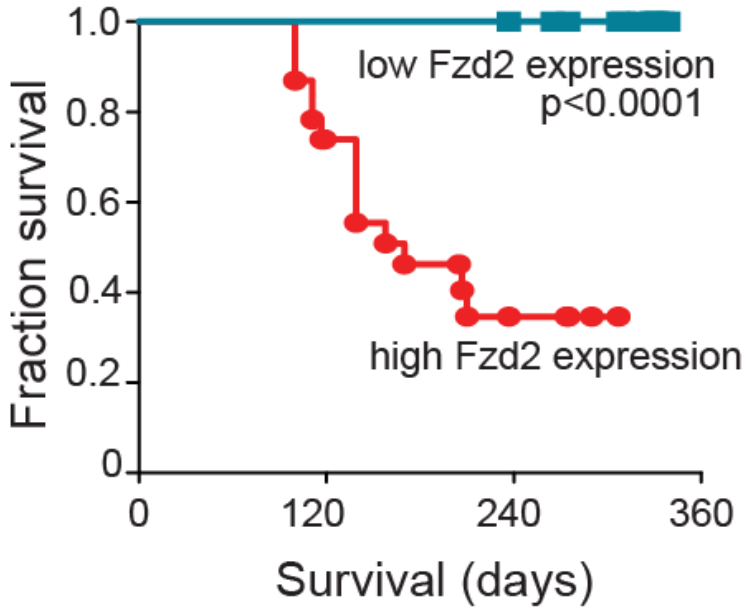
- Fzd2 expression is **high (~5 fold more) in high-grade** and **poorly differentiated** HCC tumors
- Similar effects observed in **other solid tumors** such as breast and lung

Fzd2 signaling is a strong predictor of tumor metastasis and correlates with poor overall survival (OS)

Metastasis prediction by Fzd2 expression in 46 patients with early and late-stage HCC



OS and Fzd2 expression in 46 patients with early and late-stage HCC



- **Fzd2** expression only can **predict tumor metastasis** with **>80% accuracy**
- **High Fzd2** expression **correlates** with **poor overall survival**
- **Similar effects** observed in **other solid tumors** such as breast and lung

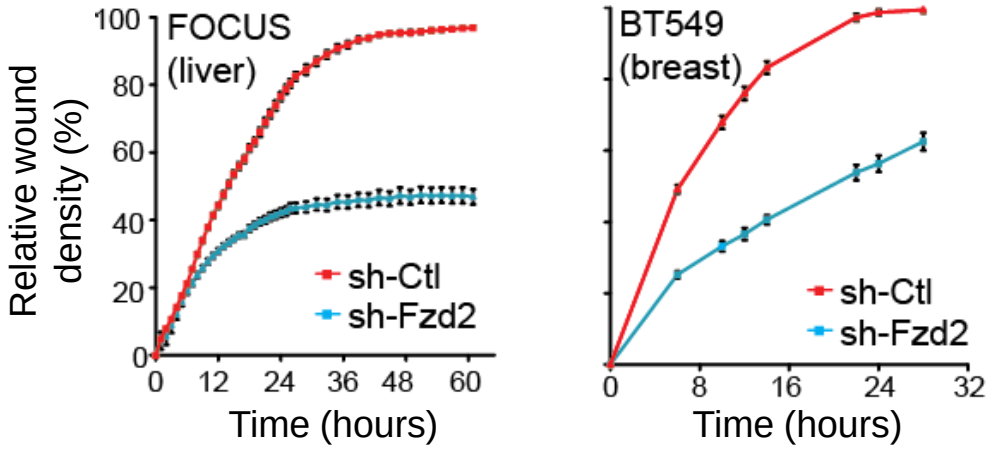
Correlation vs. Causation

- Wnt5-Fzd2 **correlates** with
  - differentiated tumors
  - Metastasis
  - poor OS

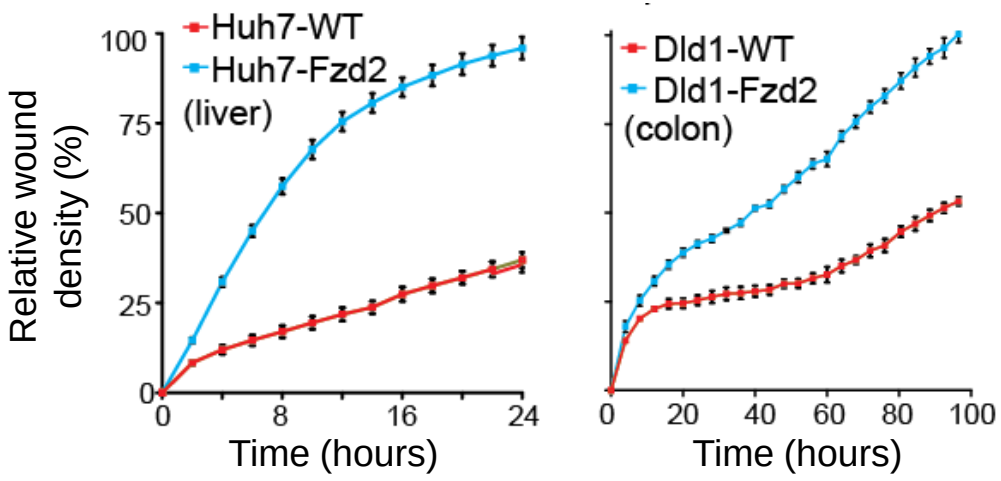
- Does Wnt5-Fzd2 **drive** these processes? How?

Wnt5-Fzd2 drives cell migration

Effect of Fzd2 knockdown on cell migration



Effect of Fzd2 overexpression on cell migration



- Fzd2 **knockdown decreases cell migration** in poorly differentiated FOCUS cells
- Fzd2 **overexpression promotes cell migration** in well differentiated Huh-7 cells
- Effects are **independent** of canonical (**β-catenin**-mediated) signaling

RPPA application for dissecting signaling pathways

Transfect cells with siRNA's or cDNA's

Collect time points of Wnt stimulation → lysates

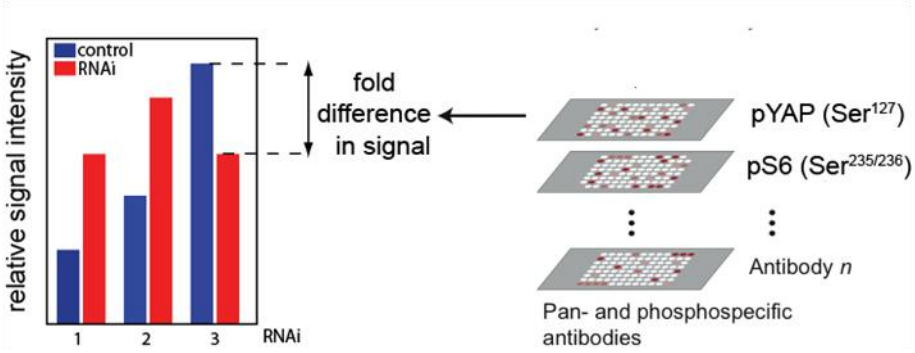
Filter clear, protein quant.

Microarray lysates

Aushon 2470

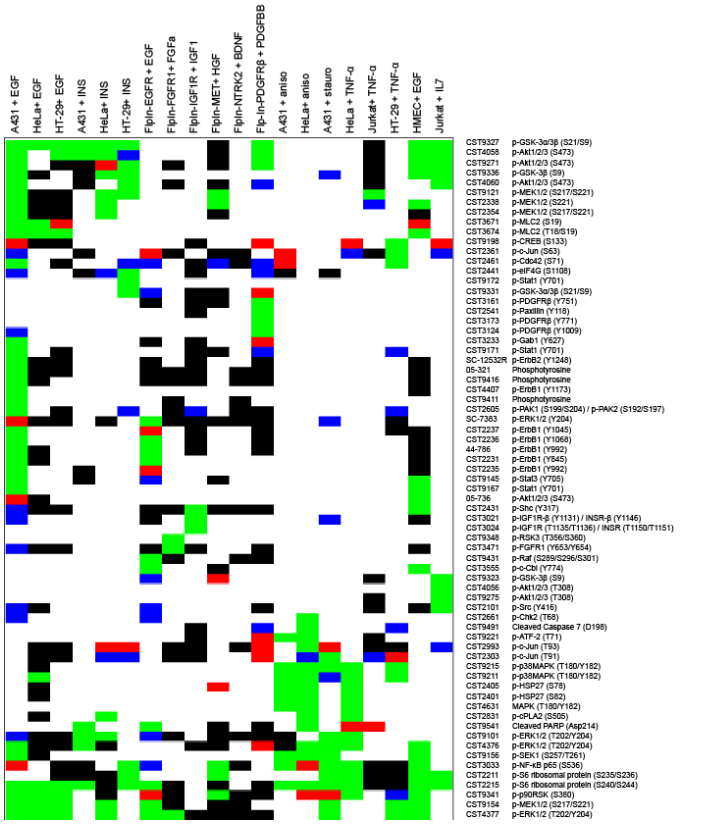
Probe and Scan slides

InnoScan 710-IR



Luckert & Gujral *et al.*, Science Signaling. 2012

We have screened >600 antibodies from commercial sources



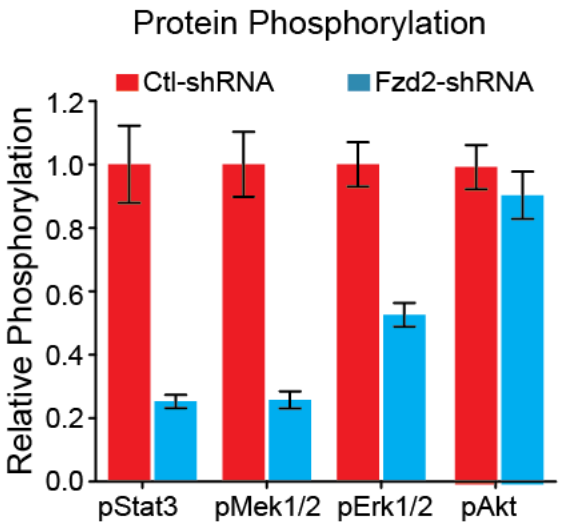
Sevecka *et al.*, Mol Cell Proteomics. 2011

>100 validated antibodies (context specific)

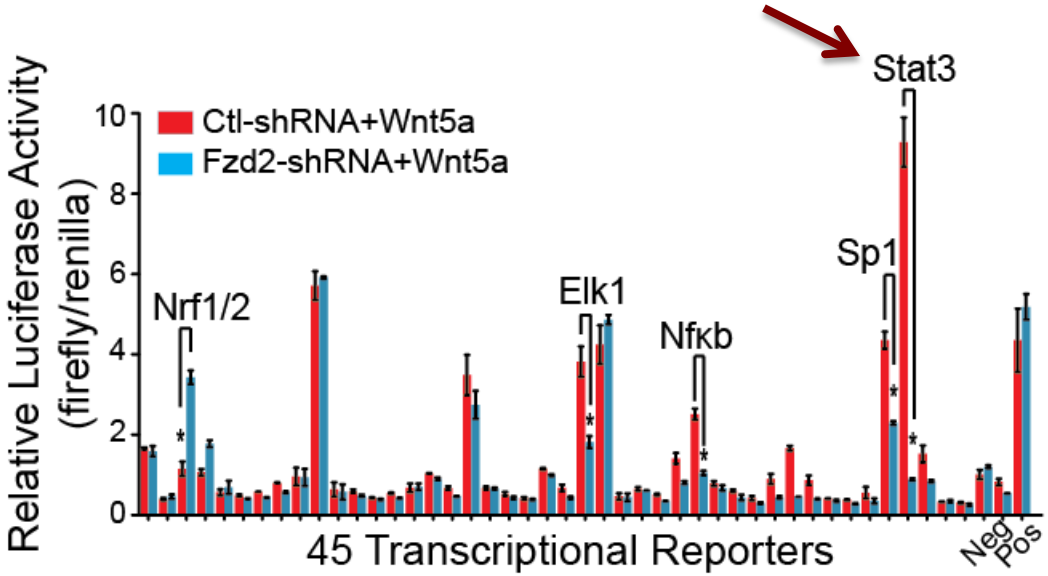
- **Validated antibodies applied to:**
  - **Wnt signaling** (Luckert & Gujral *et al.*, Science Signaling, 2012)
  - **DNA damage response** (Lee *et al.*, Cell, 2012)
  - **Malaria parasites** (Kaushansky *et al.*, Cell Rep, 2013)
  - **Breast tumor biopsies** (Gujral *et al.*, Oncogene, 2013)
  - **RTK Signaling** (Wagner JP et al, Science Signaling, 2013)

Fzd2 signals through MAPK and Stat3

- Fzd2 knockdown decreases activation of the MAPK pathway and Stat3

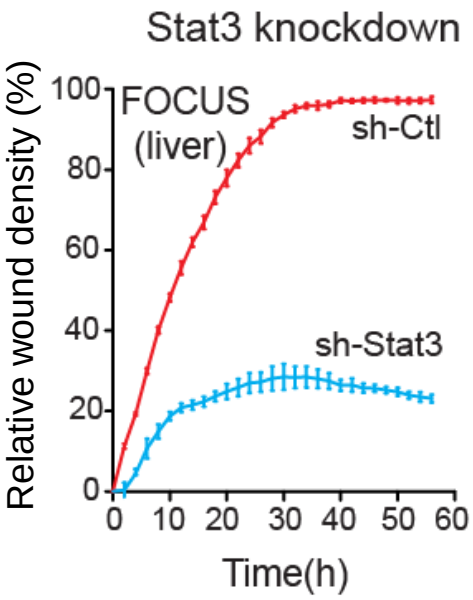


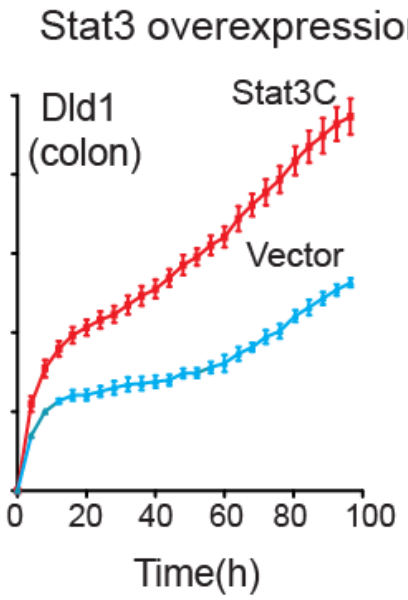
- Fzd2 knockdown decreases Stat3-mediated transcription

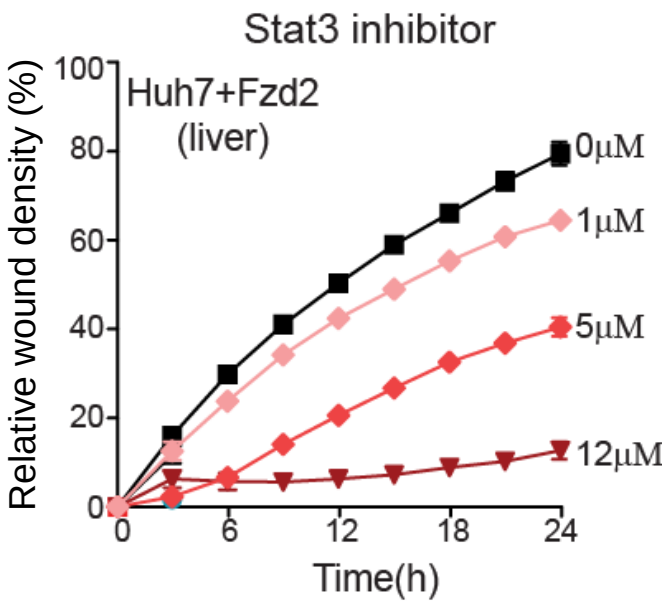


Fzd2 mediates migration through Stat3

- Stat3 knockdown decreases migration
- Stat3 overexpression promotes migration
- Stat3 inhibitor decreases migration in a dose-dependent manner







Pathway revealed through pharmacogenomics

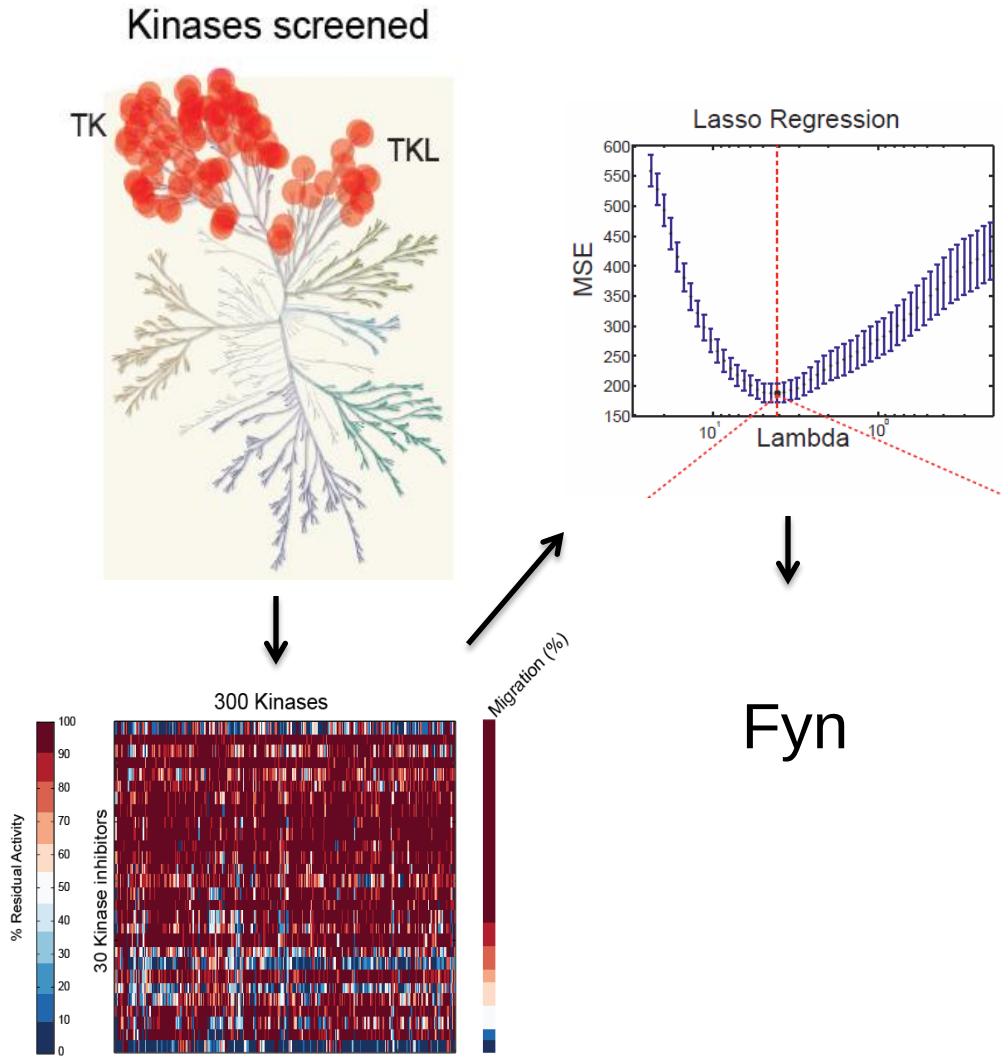
Wnt5a/b

Fzd2

?

Stat3

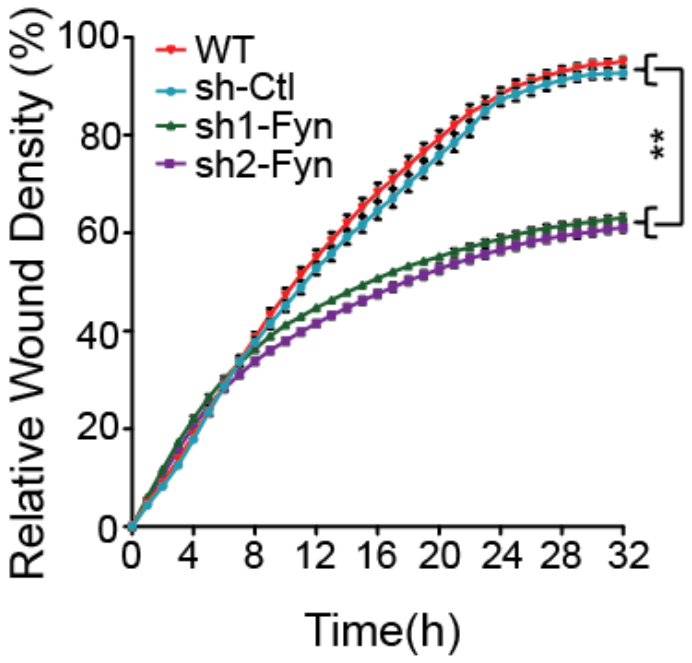
Cell migration



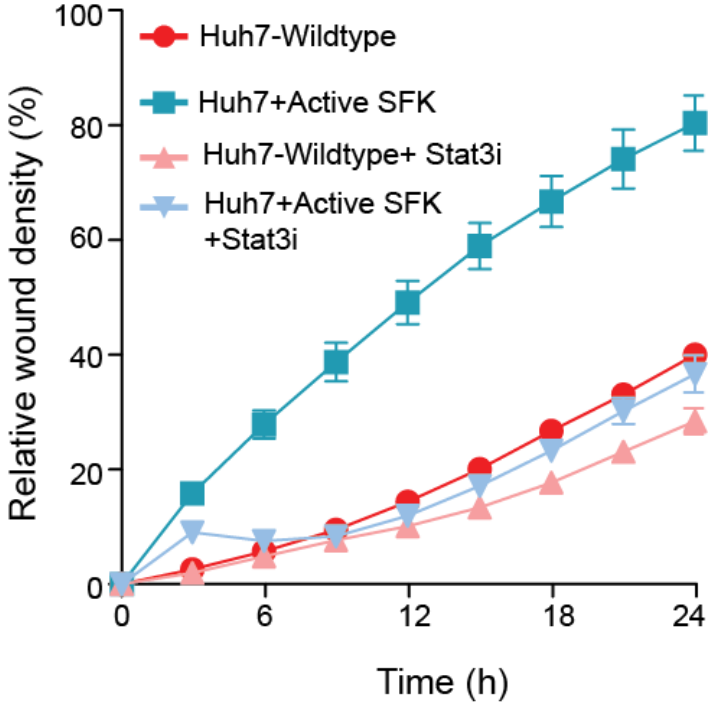
Proc Natl Acad Sci USA (2014) **111**, 5048

Fyn Kinase: a key mediator of Fzd2 signaling and cell migration

- Fyn knockdown decreases cell migration

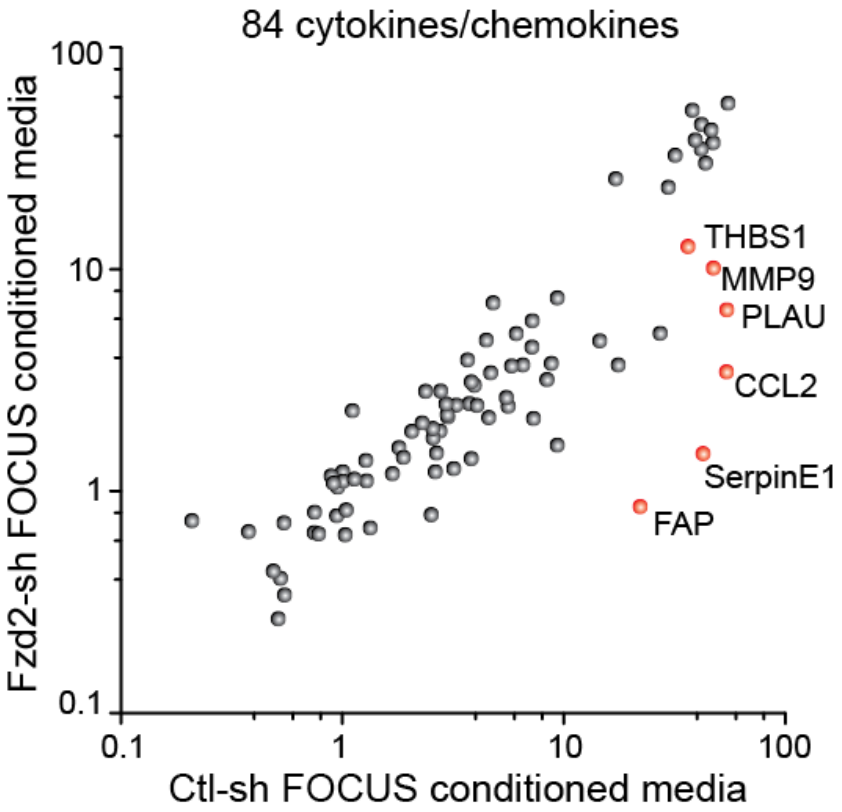


- Active Fyn promotes cell migration in a Stat3-dependent manner

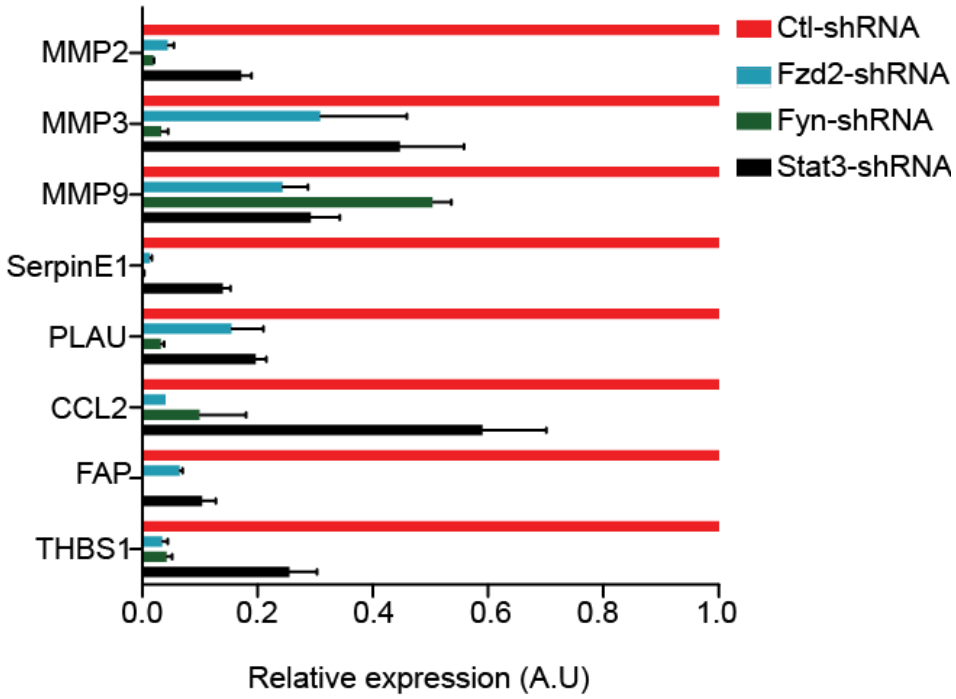


Fzd2 induces expression and release of metastasis-promoting factors

- Fzd2 induces secretion of pro-metastatic cytokines

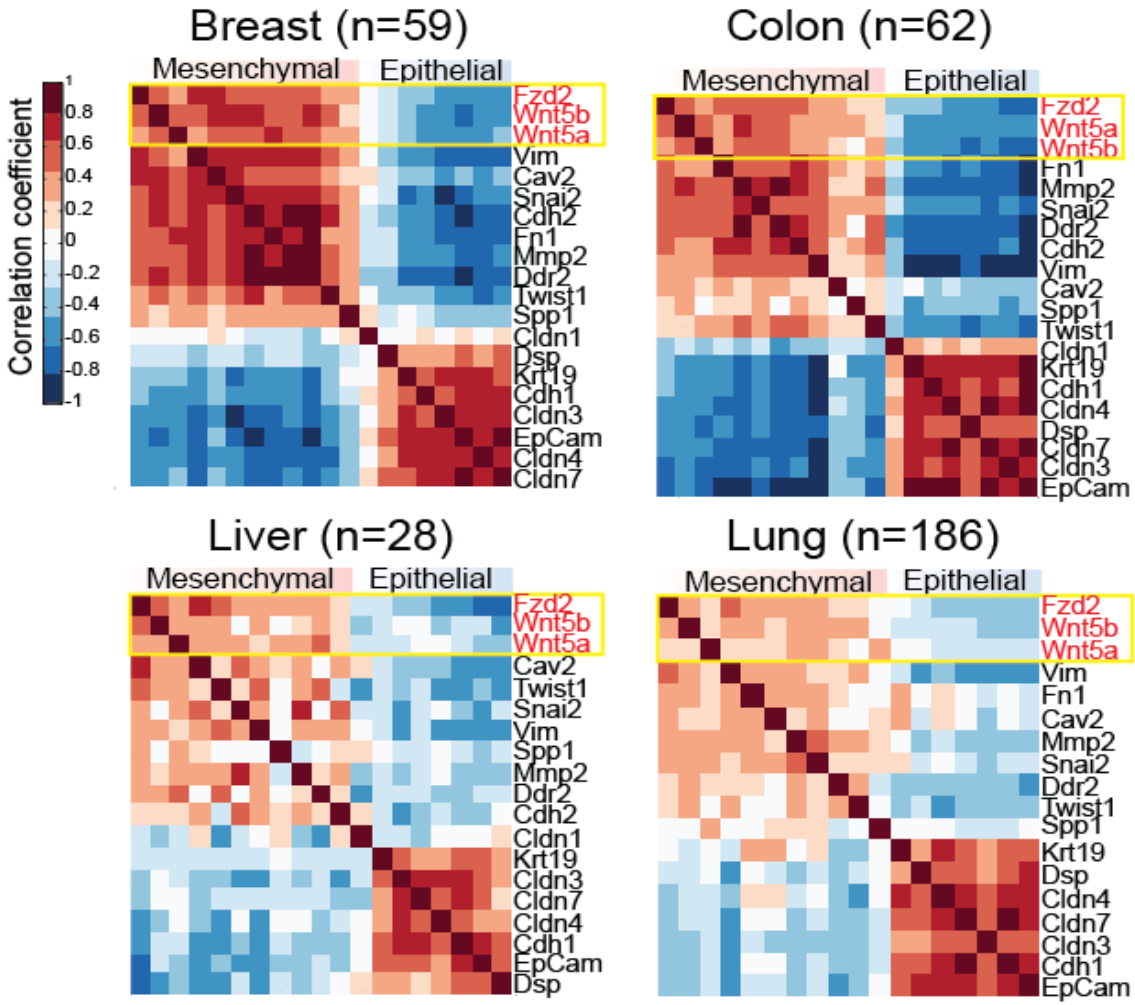


- Cytokine release depends on Fzd2, Fyn, and Stat3



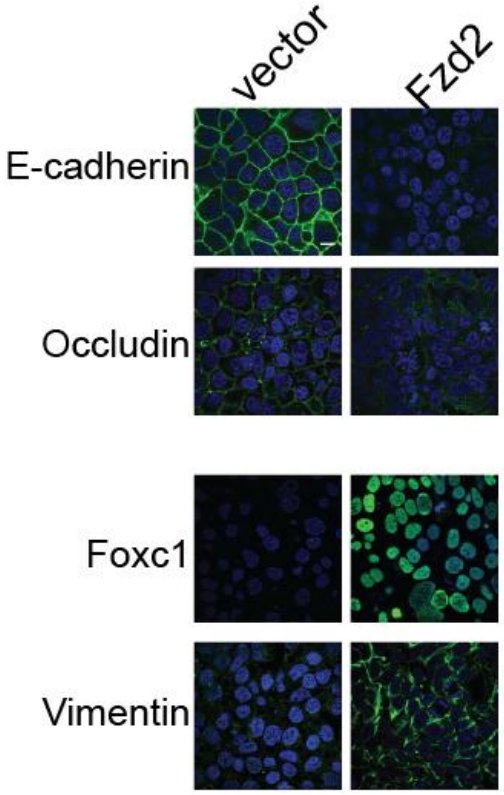
Fzd2 correlates with markers of epithelial-mesenchymal transition (EMT)

mRNA levels of Fzd2 and EMT markers in solid tumors

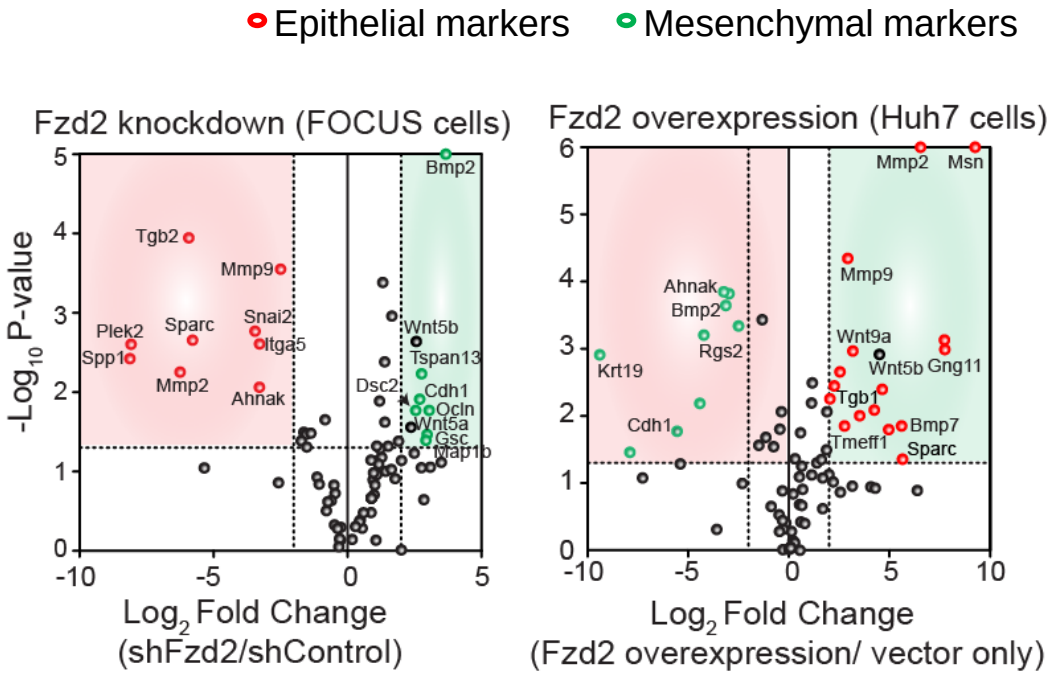


Fzd2 promotes EMT

Effect of Fzd2 overexpression on cell adhesion proteins in Huh 7 cells



Effect of Fzd2 levels on mRNA expression of EMT gene signature

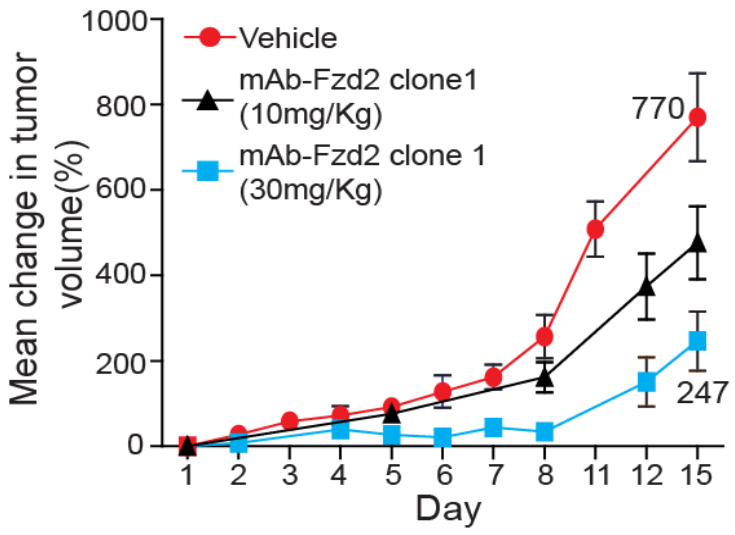


- **Fzd2 overexpression decreases** the membrane expression of **cell adhesion proteins** while **increasing** the protein expression of **mesenchymal markers**
- Changes in **Fzd2** expression levels **modulate** the expression levels of **EMT gene signature**

Anti-Fzd2 mAbs inhibit tumor growth and metastasis

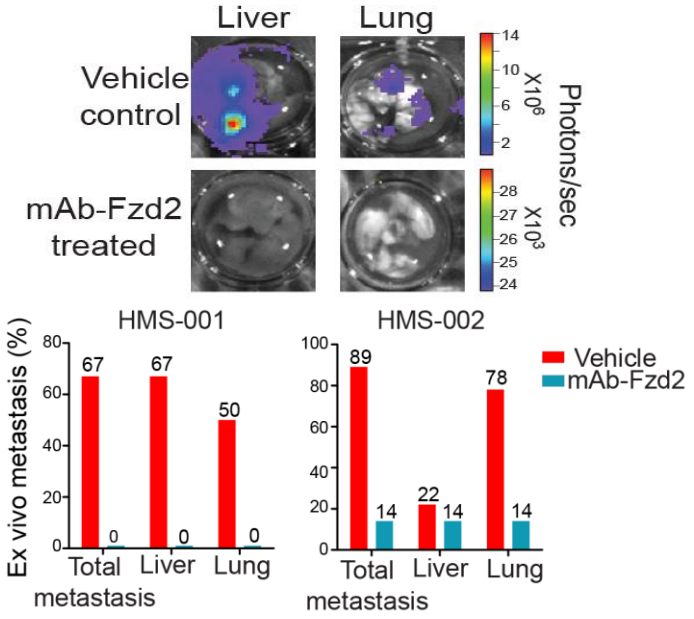
Effect of mAb-Fzd2 on tumor growth in mouse xenografts

% change in tumor volume



Effect of mAb-Fzd2 on tumor metastasis in mouse xenografts

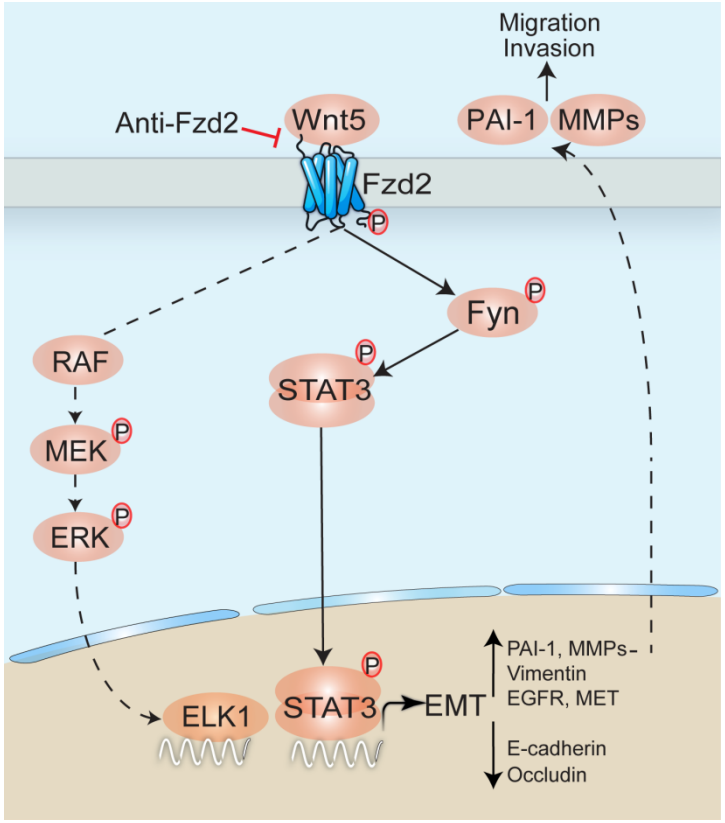
% of mice with liver or lung metastases



- **mAb-Fzd2 slows down** (>3 fold) **tumor growth** in a **dose-dependent** manner
- **mAb-Fzd2 prevents tumor metastasis** to liver and lung
  - **Metastases** were detected in **~67% (4/6)** and **~89% (8/9) of control mice** vs **0% (0/5)** and **14% (1/7) of mice treated with mAb-Fzd** in two independent studies, respectively

Summary

Non-canonical Fzd2 Signaling



- **Fzd2** and its ligands, **Wnt5a/b**, are **upregulated** in late-stage, **metastatic cancers**
- **Fzd2** is **prognostic** of **poor overall survival** in HCC
- **Fzd2 drives tumor growth**, **EMT** and **metastasis** through a newly discovered **noncanonical Wnt signaling** pathway
- **Anti-Fzd2** monoclonal **antibodies inhibit cell migration** *in vitro* and both **tumor growth** and **metastasis** in mouse xenograft models