Therapy-Induced Rewiring — Tutorial Overview

Aim

This notebook applies the **SpatialMMKPNN framework** to analyze therapy-induced rewiring of tumor–stroma–immune communication. Using paired samples from **GSE238264 (HCC, neoadjuvant cabozantinib + nivolumab)**, the workflow quantifies how **ligand–receptor (LR) signaling axes relocate spatially** and change in magnitude after therapy.

The emphasis is on **interpretable, mechanism-first questions**:

- Where do specific signaling axes move after therapy?
- How much do they expand or contract in absolute terms?
- Are observed relocations internally consistent across measures and patients?

What This Notebook Shows

- Construction of paired pre- and post-therapy LR networks.
- **Within-Axis Spatial Redistribution (WASR):** measures where an axis moves (boundary vs interior; distance bins).
- **Magnitude (Δedges):** measures how much an axis expands or contracts.
- Joint interpretation: “where” + “how much” gives a full rewiring narrative.
- Internal robustness: agreement across Δshare and Δedges, boundary vs distance-bin consistency, and per-patient directionality.
- Outputs: key CSV summaries and plots for reproducibility.

Key Design Choices (the “why”)

- **Within-axis denominator:** avoids compositional artifacts across axes.
- **Parallel magnitude reporting:** complements Δshare with absolute Δedges.
- **Boundary definition:** first distance bin per slide = boundary (robust across scales).
- **Patient-specific reporting:** therapy effects are idiosyncratic, not pooled.

- **Conservative endpoint claims:** role-mapping heuristics are kept cautious until QC is stricter.

Method (step-by-step)

Dataset

- **Source:** GSE238264 hepatocellular carcinoma slides.
- **Patients:** PT01–PT03 with paired pre- and post-therapy samples.
- **Therapy labels:** provided in `therapy_labels_GSE238264.csv`.

Preprocessing

- Overlay of ligand–receptor edges (`marker_overlay_by_slide_GSE238264.csv`).
- Axis whitelist: therapy-relevant (TLS, vascular RTKs, myeloid) + baseline axes (TGFB1/TGFBR, CXCL12/CXCR4, SPP1/integrin, VEGFA/KDR).

Rewiring Analysis

1. **Within-Axis Spatial Redistribution (WASR):**
    - Boundary vs interior; distance bins (quartiles).
    - Output: Δshare values (post–pre).
2. **Magnitude (Δedges):**
    - Absolute change in edge counts (post–pre).
    - Interpreted jointly with WASR.

Robustness

- Internal consistency checks only:
    - Coherence between Δshare and Δedges.
    - Agreement between boundary vs distance-bin results.
    - Per-patient directionality.
- Criteria are applied within the notebook and summarized in `rewiring_stats.csv` and `wasr_stats.csv`.

Outputs

- `rewiring_stats.csv`
- `wasr_stats.csv`
- `therapy_summary.csv`
- Plots in `/Plots/`:
  - `axis_delta_bar.png`
  - `rewiring_volcano.png`
  - `volcano_right_labels.png`
  - `volcano_with_table.png`

Additional intermediate files (deltas, patient-level breakdowns, distance-bin tables) are recomputed by rerunning the notebook.

Results (Therapy-Induced Rewiring Summary)

- **PT02:** VEGFA–KDR relocated inward (Δshare +0.164 interior) with expansion inside (+105k edges) and loss at boundary (–16k). SPP1–integrin concentrated at the rim (+0.145 boundary share) while expanding overall.
- **PT03:** CXCL12–CXCR4 shifted inward (+0.140 interior share) with interior growth (+23k edges). VEGFA–KDR also showed interior bias.
- **PT01:** TGFB1–TGFBR contracted overall (Δedges negative) but skewed interiorly (+0.084 interior share).
- Together, these show **spatial rewiring** rather than simple up/down scaling. Distance-bin views corroborated boundary/interior narratives.

Robustness:

- **Boundary vs interior:** 20/24 strata passed the notebook-defined criteria (~83%).
- **Distance bins:** 34/48 strata passed the notebook-defined criteria (~71%).
- Coherence between Δshare and Δedges supports that these shifts represent true rewiring rather than artifacts.

  Note on Outputs
  This repository includes only the key summary CSVs and plots needed to reproduce the main findings. The notebook itself contains instructions and code cells to generate all intermediate files (per-patient deltas, boundary vs interior tables, distance-bin breakdowns) and to display detailed results in the console. Users who need the full set of artifacts can simply rerun the notebook end-to-end.

Limitations & Recommendations

- **Limited sample size:** results are based on three patients with paired pre/post samples. Robustness analyses provide internal consistency but do not substitute for larger cohorts.
- **No resampling/null models:** robustness checks do not include bootstraps or randomized nulls; these would be a future extension.
- **Pixel-based bins:** WASR bins are distance quantiles, not fixed µm; robust for within-slide comparisons but harder to generalize across slides.
- **Whitelist supervision:** focusing on therapy-relevant axes improves interpretability but may miss unanticipated signals.
- **Role assignment heuristics:** tumor/non-tumor roles were inferred where annotations were missing.

Recommendations:

- Validate rewiring signals in larger, independent cohorts.
- Consider adding resampling or null models in future extensions to strengthen robustness.
- Where possible, convert WASR bins to physical units for cross-slide comparison.
- Supplement whitelist analysis with unsupervised ranking of all LR pairs.

Interpretation

This application shows that **therapy rewires tumor signaling spatially**: axes move to new niches before their overall magnitude changes.

- VEGFA–KDR and CXCL12–CXCR4 gain **interior bias**.
- SPP1–integrin strengthens a **boundary motif**.
- TGFB contracts yet shifts interiorly.

  The **inward relocation of VEGFA–KDR** signals suggests that therapy may not eliminate angiogenic signaling but rather **shifts it toward the tumor core**, potentially supporting vascular remodeling in protected niches.

  The **CXCL12–CXCR4 inward bias** is consistent with chemokine-driven **immune cell sequestration** inside the tumor, a mechanism often linked to **immune evasion**.

  The **SPP1–integrin boundary motif** aligns with a stromal barrier phenotype, where adhesion

and ECM remodeling at the rim may **stiffen the boundary and resist immune infiltration**.

The **TGFB contraction yet interior skew** indicates that therapy reduces overall TGFB signaling but preserves or accentuates its effects **within the tumor compartment**, a pattern that could contribute to **adaptive resistance**.

Together: these patterns show that therapy does not simply “upregulate” or “downregulate” signals — it **repositions communication axes** across spatial niches, reprogramming the microenvironment in ways that influence angiogenesis, immune exclusion, and stromal barriers.