Elucidating the relationship between H3K36 and H3K27 trimethylation across normal and malignant tissue types

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Histone H3K27 and H3K36 trimethylation in non-malignant cells

- H3K27me3 and H3K36me3 are typically associated with **transcriptional repression** and **activation**, respectively, and usually exhibit mutual exclusivity.

- Increasing evidence of crosstalk between H3K36me2/3 and H3K27me3 modifiers:
  - H3K36me3 shown to inhibit PRC2 subunit EZH2, preventing the spreading of H3K27me3.
  - Conversely, PRC2 subunit PHF19 may recruit H3K36me3 demethylase NO66 to H3K36me3 sites.

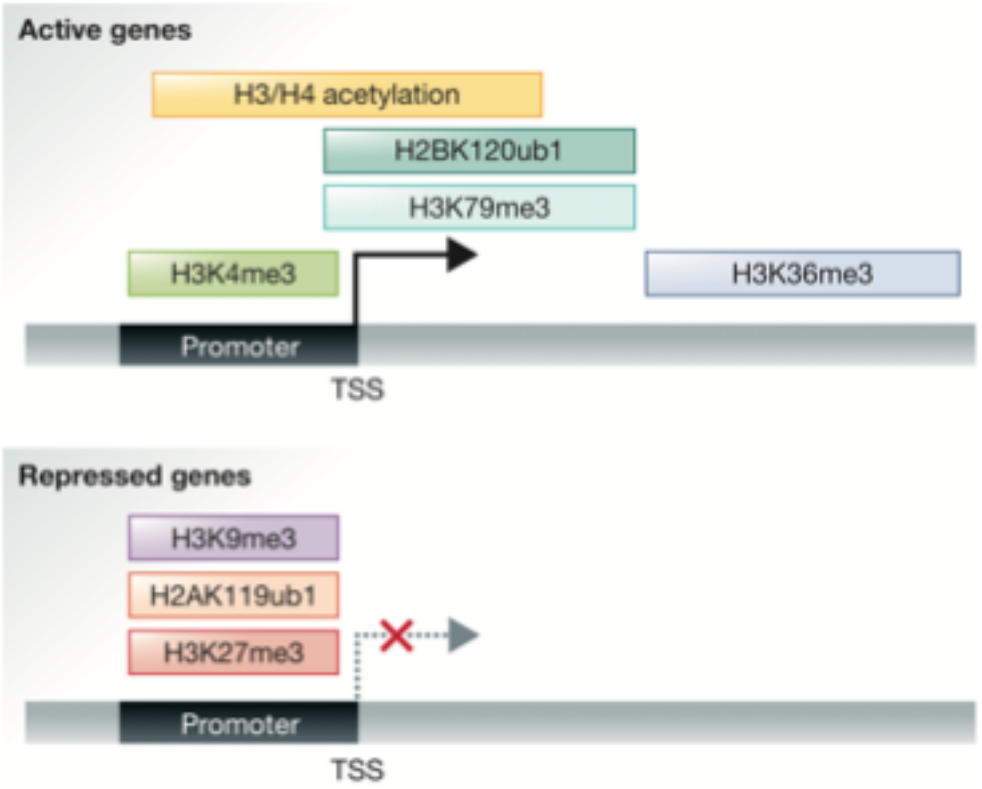


Fig 1. Conventional distribution of H3K36 and H3K27 trimethylation marks.

Zhang *et al.*, EMBO rep. 2015

Redistribution of H3K27me3 and HeK36me3 in cancer

- Cancer-associated H3 (K36M) mutations co-localize with sites of H3K27 and H3K36 methylation and alter typical binding patterns.
- Disruptions to H3K36 methylation cause depletion of H3K36me3 and expansion of H3K27me3.
  - Impairs differentiation of mesenchymal progenitor cells and is sufficient for sarcoma tumorigenesis.
- Interplay of H3K27me3 and H3K36me3 seems to have an oncogenic role in pediatric cancers.

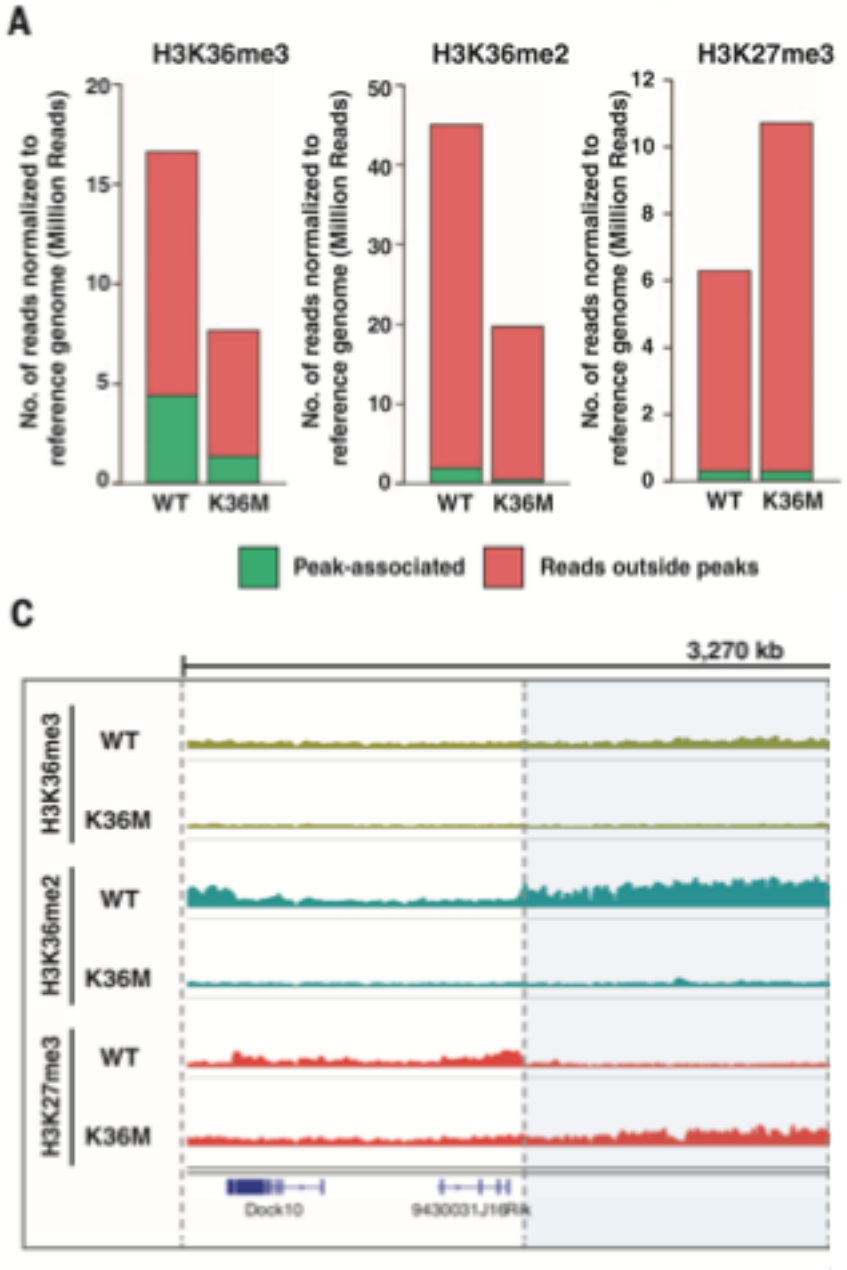


Fig 2. Genome-wide depletion of H3K36me3 allows for expansion of H3K27me3.

Lu *et al.*, Science 2016

Elucidating the relationship between H3K36 and H3K27 trimethylation across normal and malignant tissue types

Aim: to characterize the relationship between H3K36 and H3K27 trimethylation in cancer and clarify its possible oncogenic role.

- Exploratory analysis across multiple datasets comprising 110 paired samples:
  - 91 from Centre for Epigenome Mapping Technologies (**CEMT**) consortium (normal + tumor samples).
  - 15 from NCI Rhabdoid tumor project (tumor, cell lines, embryonic stem cells).
  - 4 small cell carcinoma of the ovary hypercalcemic type (**SCCOHT**) tissues.

- Whole-genome H3K36me3 and H3K27me3 ChIP-Seq data available for all samples.

Analysis methodology

- Bam files representing raw hg19 aligned reads from H3K27me3 and H3K36me3 ChIP-Sequencing is converted to fixed-step Wig files.
- Normalized coverage in select regions (TSS +/- 2kb) is calculated and returned as a bed file.
- Coverage data for each sample is merged on genomic coordinate to produce a data matrix.
- Amalgamation of meta-data and clarifying missing fields (ongoing)

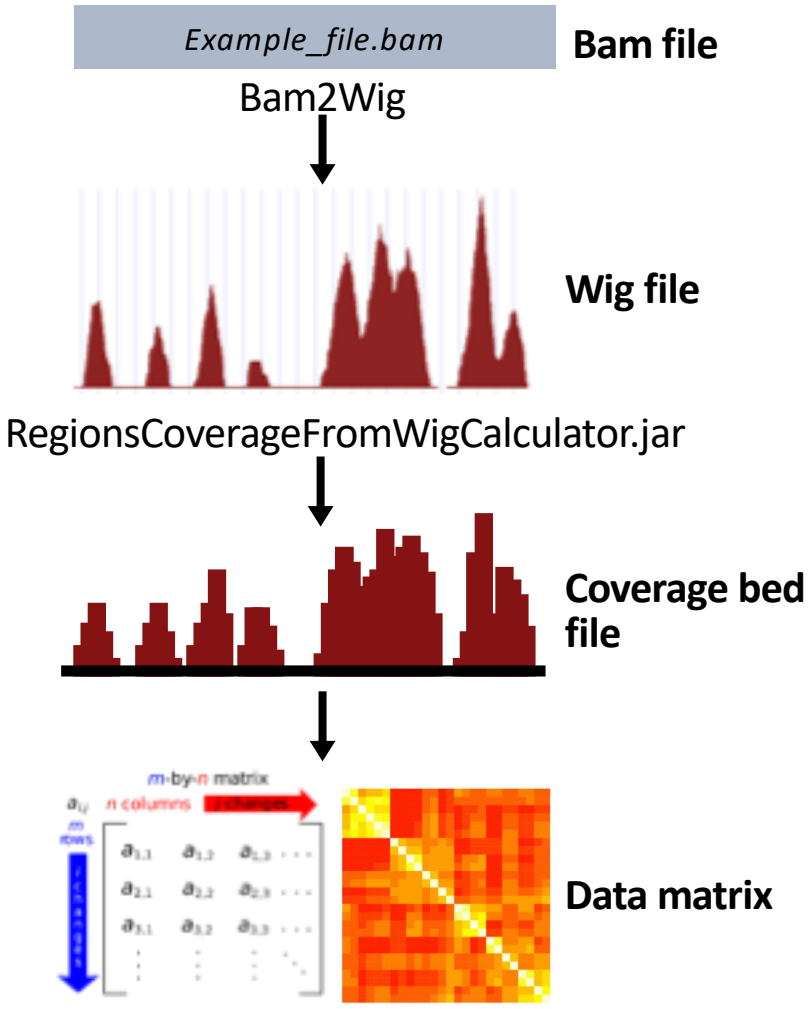


Fig 3. Schematic of analysis pipeline.

Evidence of H3K36me3 and H3K27me3 mutual exclusivity in normal cells

- Initial focus on the TSS regions of *HOX* gene clusters in non-malignant cell types.
    - HOX genes are often repressed in terminally differentiated cells.
- Normal cells are enriched for H3K27me3 and lack H3K36me3, and generally segregate according to histone marker.
- Confirmation / quality-check of anticipated behaviour in normal cells.

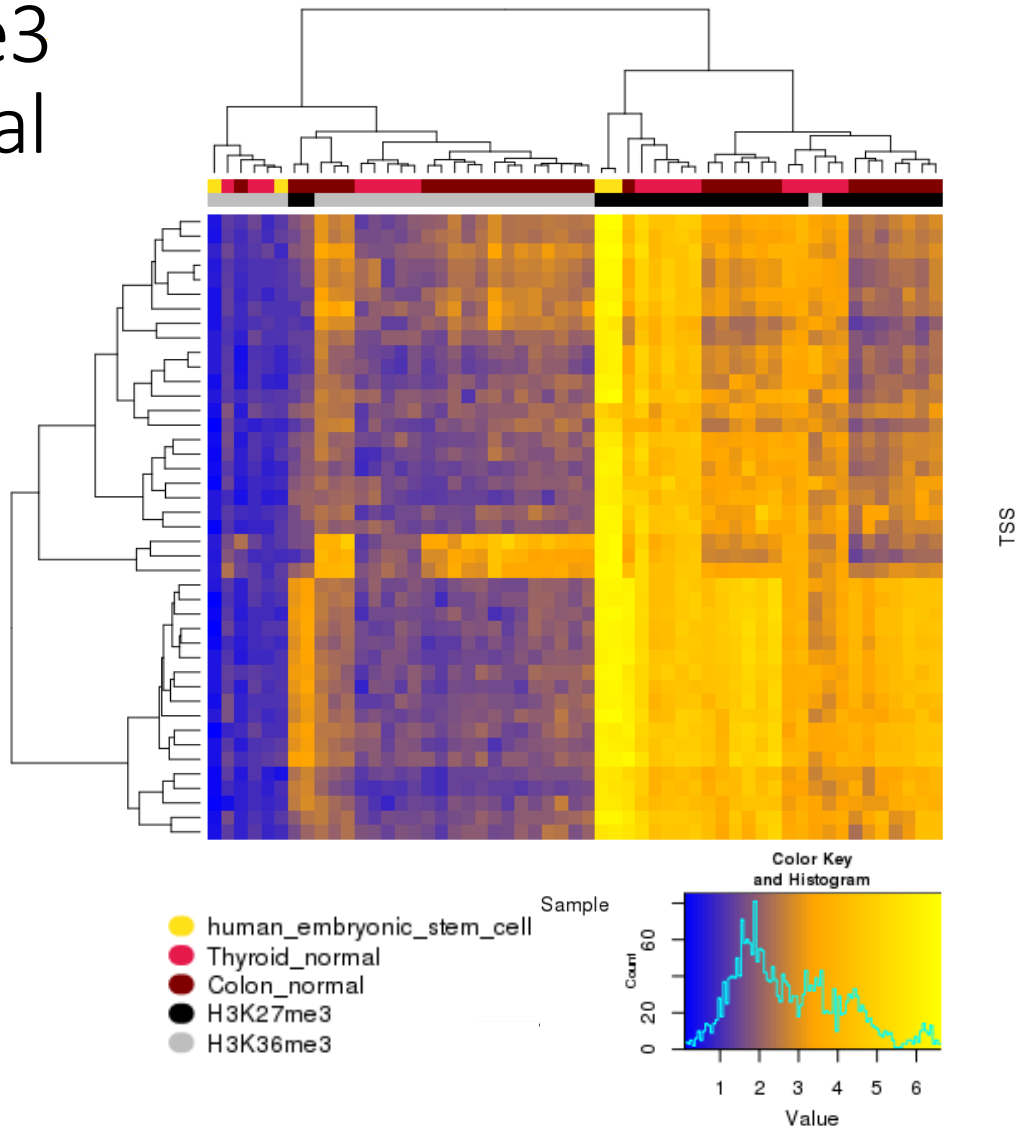
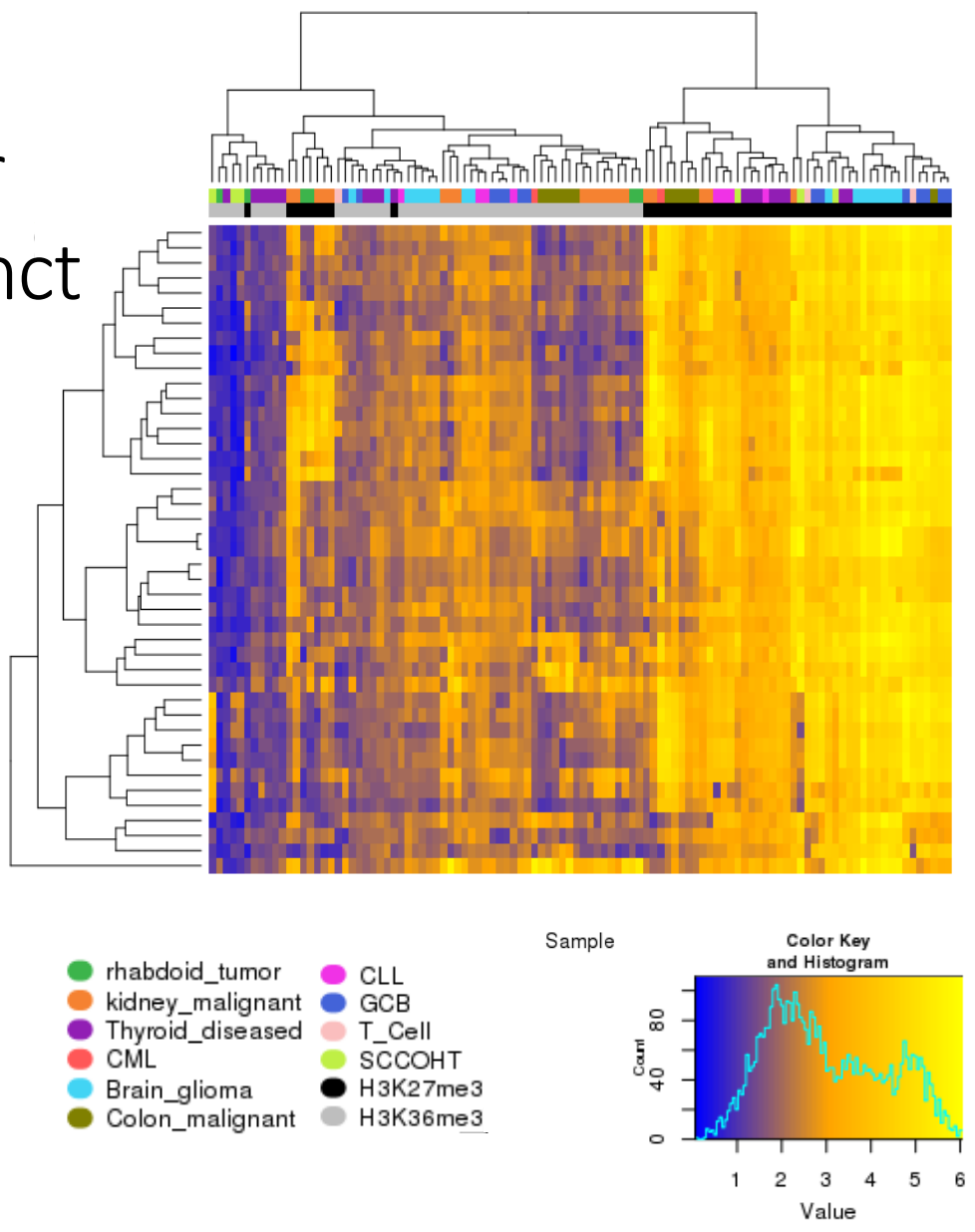


Fig 4. H3K36me3 and H3K27me3 coverage heatmap in normal cells.

Distribution of H3K36me3 and H3K27me3 in tumour cell-types is less distinct

- Gross enrichment of H3K36me3 coverage in *HOX* genes compared to normal cells (despite similar clustering behaviour).
- Initial evidence of a shift in distribution between active and repressive histone marks.

Fig 5. H3K36me3 and H3K27me3 coverage heatmap in tumour cells.



Distribution of H3K36me3 and H3K27me3 in tumour cell-types is less distinct

- Pairwise correlation among tumour samples shows clear clustering based on histone marker.
- Correlation across samples representing different histone markers is less distinct in brain glioma and germinal centre B-cell (GCB) cancers.
  - H3K27M mutations and subsequent H3K36me3 depletion / H3K27me3 expansion has been observed in pediatric brain gliomas.

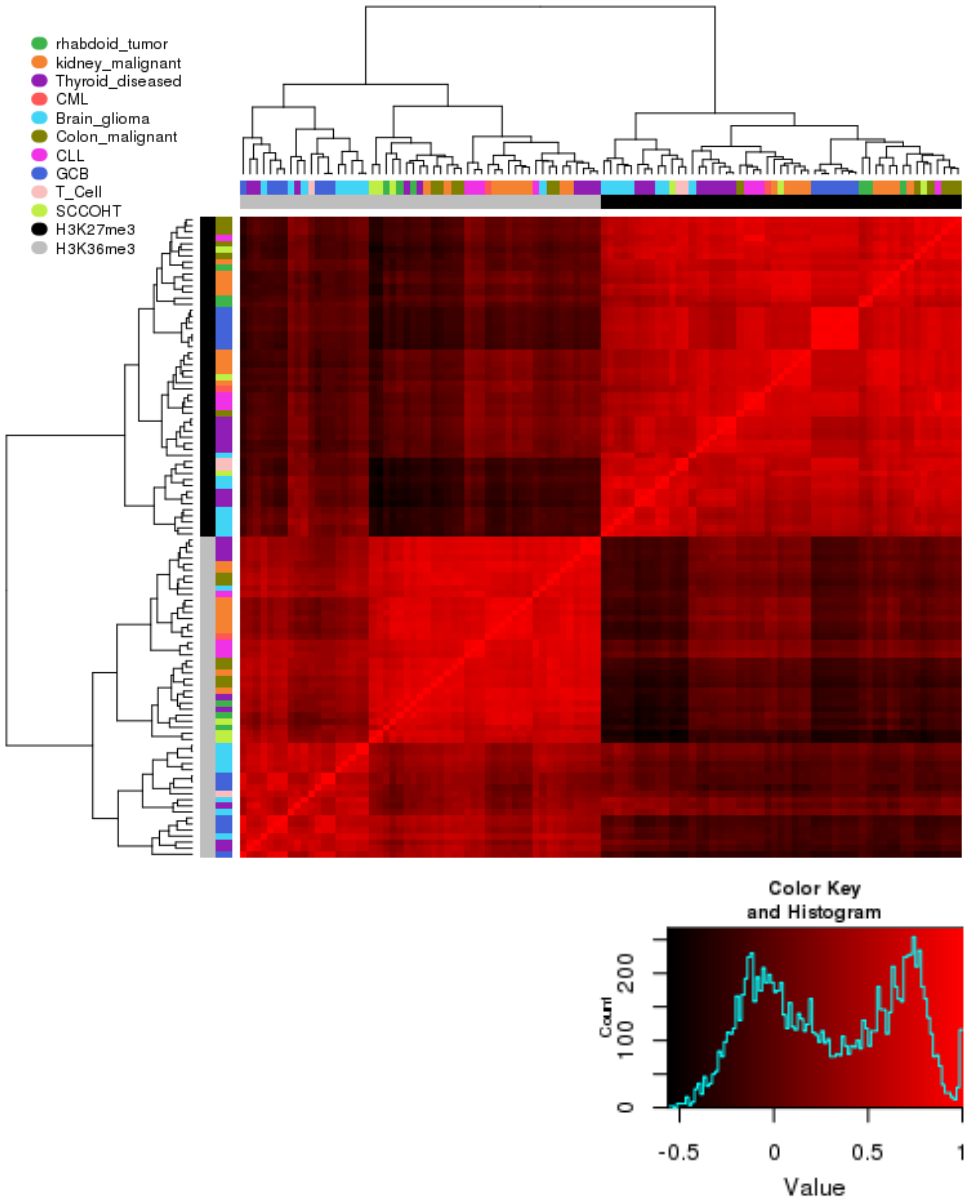


Fig 6. Pairwise genome-wide sample coverage correlation heatmap (tumour only).

Concluding remarks + future work

- H3K36me3 and H3K27me3 redistribution may have **oncogenic implications** in certain cancers.

- This is a preliminary analysis aimed at characterizing H3K36me3 / H3K27me3 histone marker distributions—leveraging a large, heterogenous collection of samples.

- Next steps: identify regions of differential H3K36me3 and H3K27me3 binding across tumor/normal samples.
    - Multiple options further exploration: Peak calling, gene-set enrichment analyses (GSEA), motif enrichment analysis, etc.

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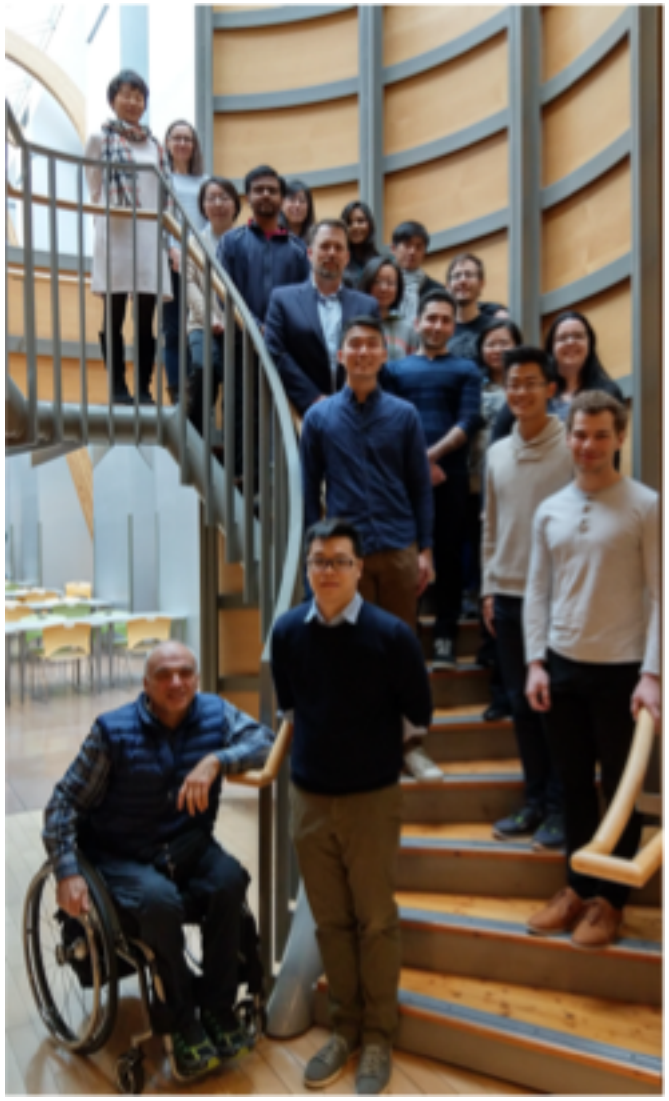

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