Genome-wide CRISPR screen reveals an uncharacterized spliceosome regulator as new candidate immunotherapy target

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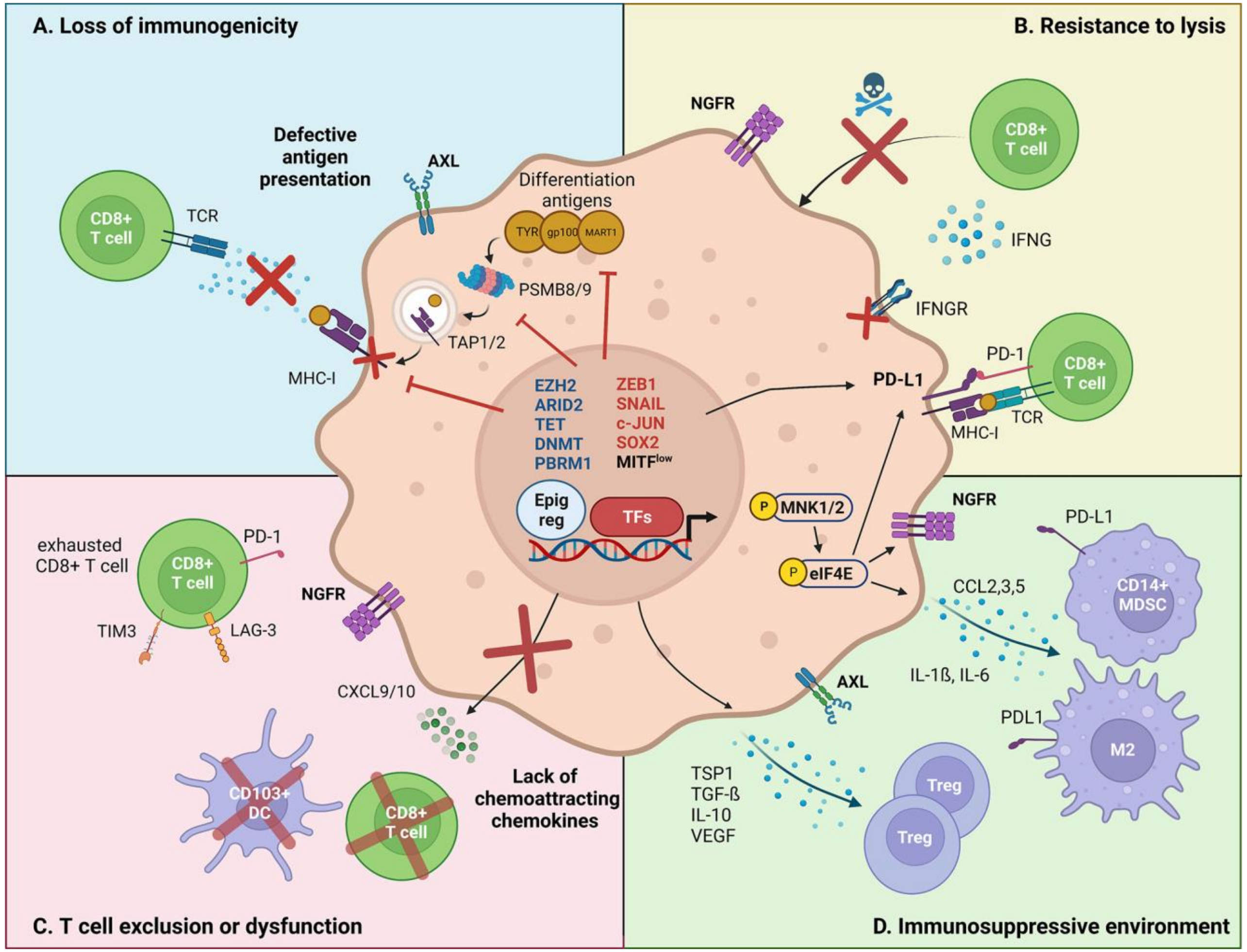
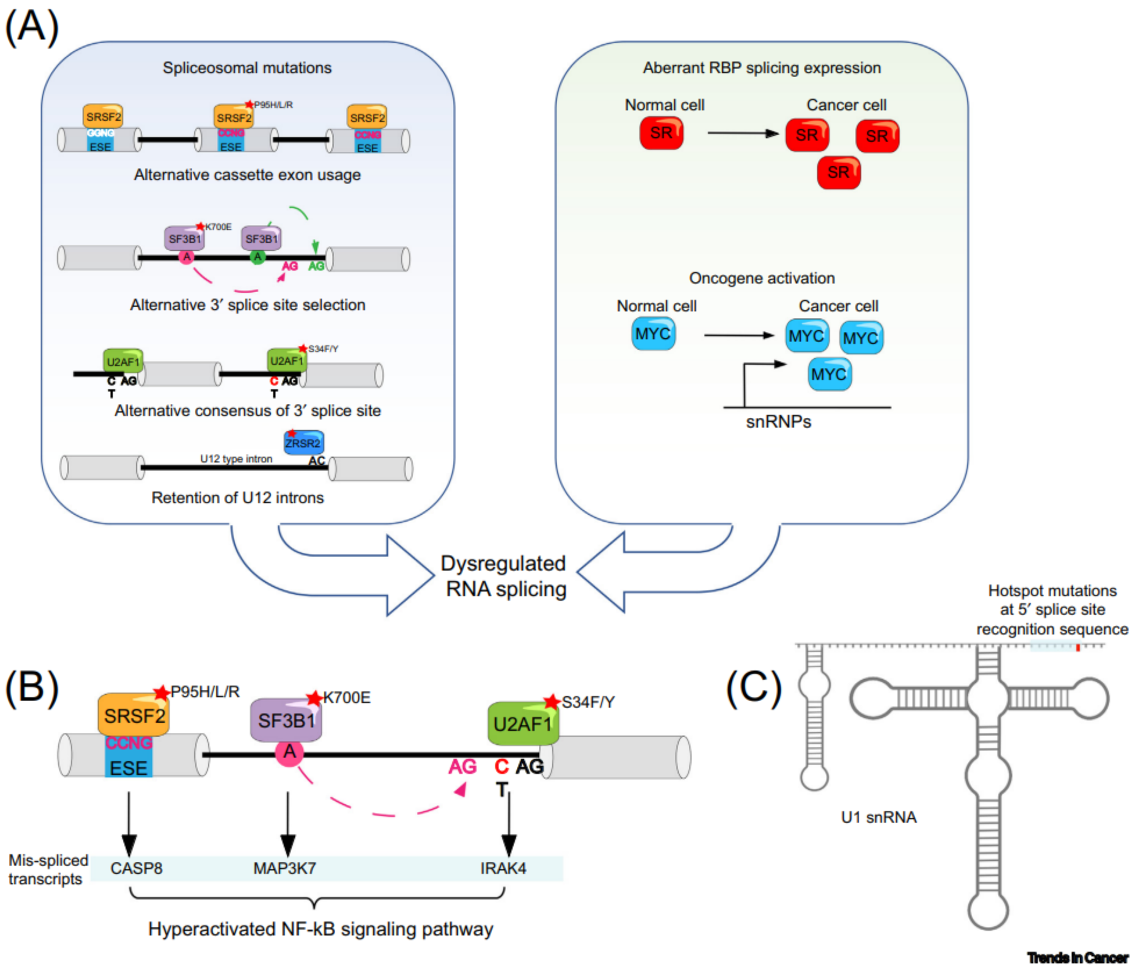
Research Background



Benboubker, Valentin, et al. "Cancer cell phenotype plasticity as a driver of immune escape in melanoma." *Frontiers in Immunology* 13 (2022): 873116.

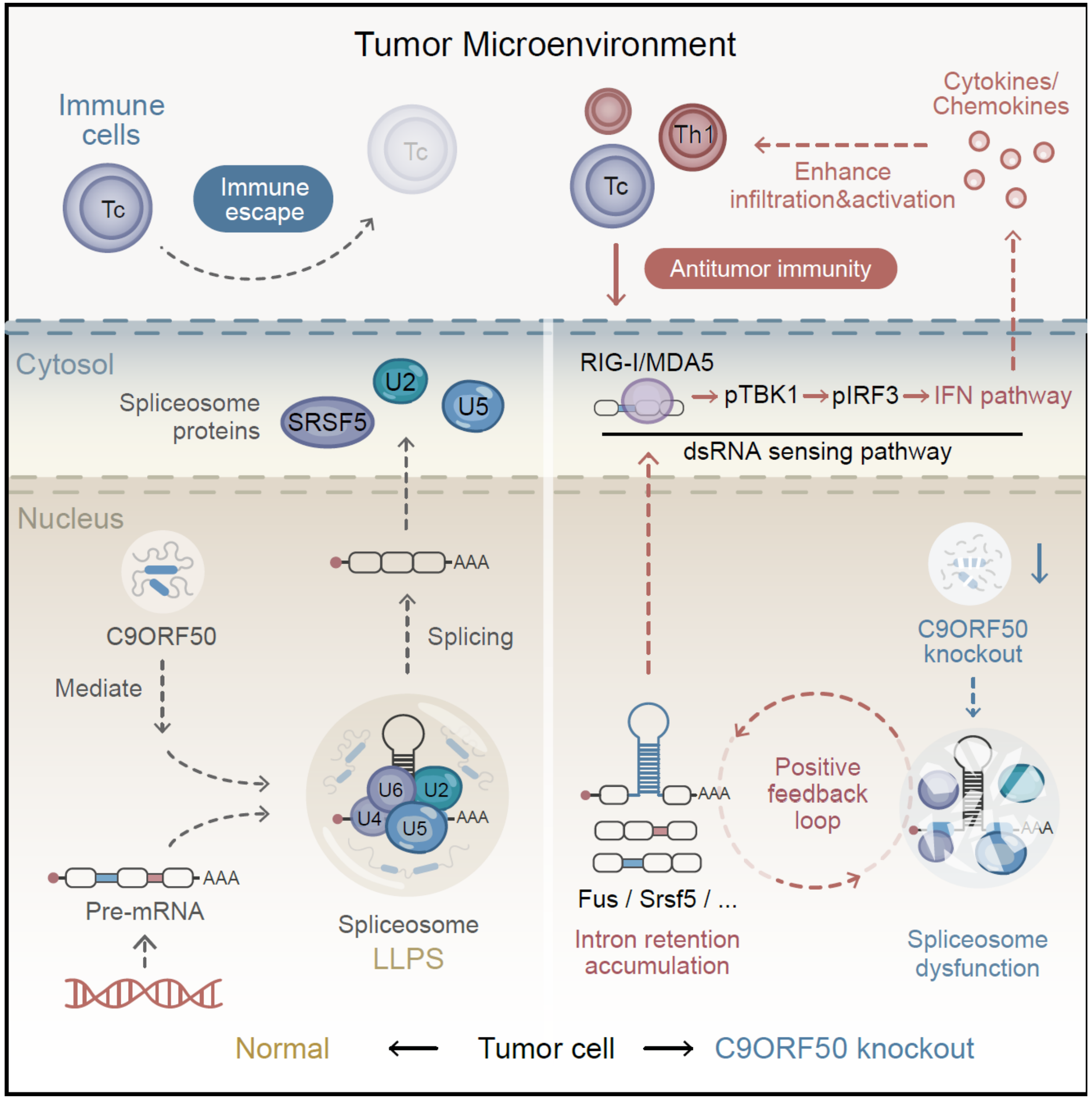


Wang, Eric, and Iannis Aifantis. "RNA splicing and cancer." *Trends in cancer* 6.8 (2020): 631-644.

Tumor cell-intrinsic mechanisms regulate immune surveillance

RNA splicing and cancer

Highlights



- An *in vivo* genome-wide CRISPR screen reveals 15 tumor-intrinsic immune evasion regulators, including an uncharacterized intrinsically disordered protein C9ORF50.
- Integrated multi-omics establishes C9ORF50 driving liquid-liquid phase separation in the nucleus, facilitating membraneless nuclear compartments formation and dynamic spliceosome organization essential for RNA processing.
- *C9ORF50* inhibition represents a potential therapeutic approach targeting a tumor-intrinsic genetic-immune axis, which induces spliceosomal intron retention, generating immunogenic dsRNA that activates type I interferon responses and $CD8^+$ T-cell recruitment, turning cold tumors hot.
- Tumor-predominant expression with non-essential roles in normal mouse physiology suggest *C9ORF50* as a candidate target warranting further safety evaluation.

Genome-scale *in vivo* CRISPR screen identifies critical drivers of cancer progression

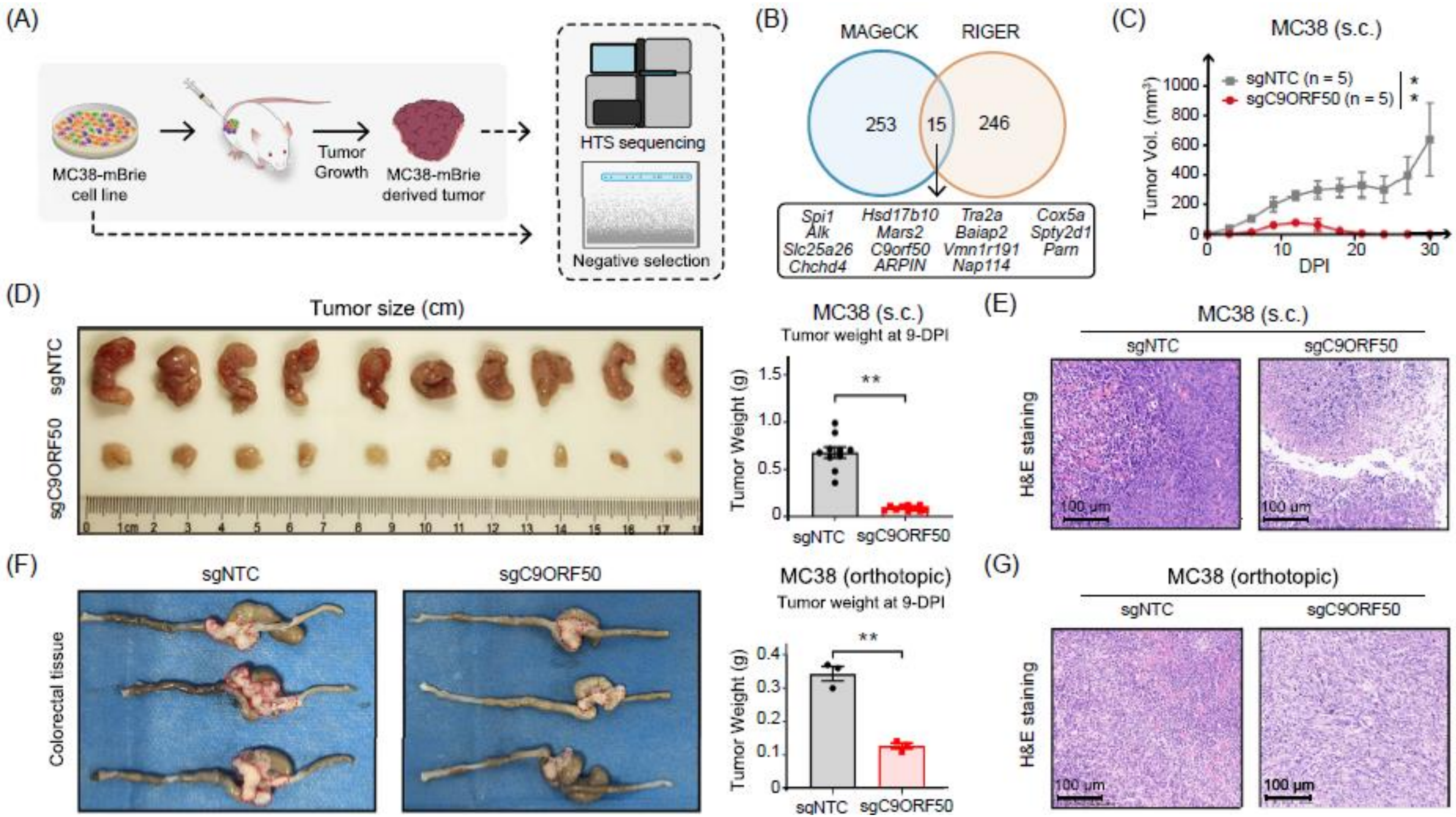


Figure 1 Genome-scale *in vivo* CRISPR screen identifies *C9ORF50* as a critical driver of cancer progression.

Genome-scale *in vivo* CRISPR screen identifies critical drivers of cancer progression

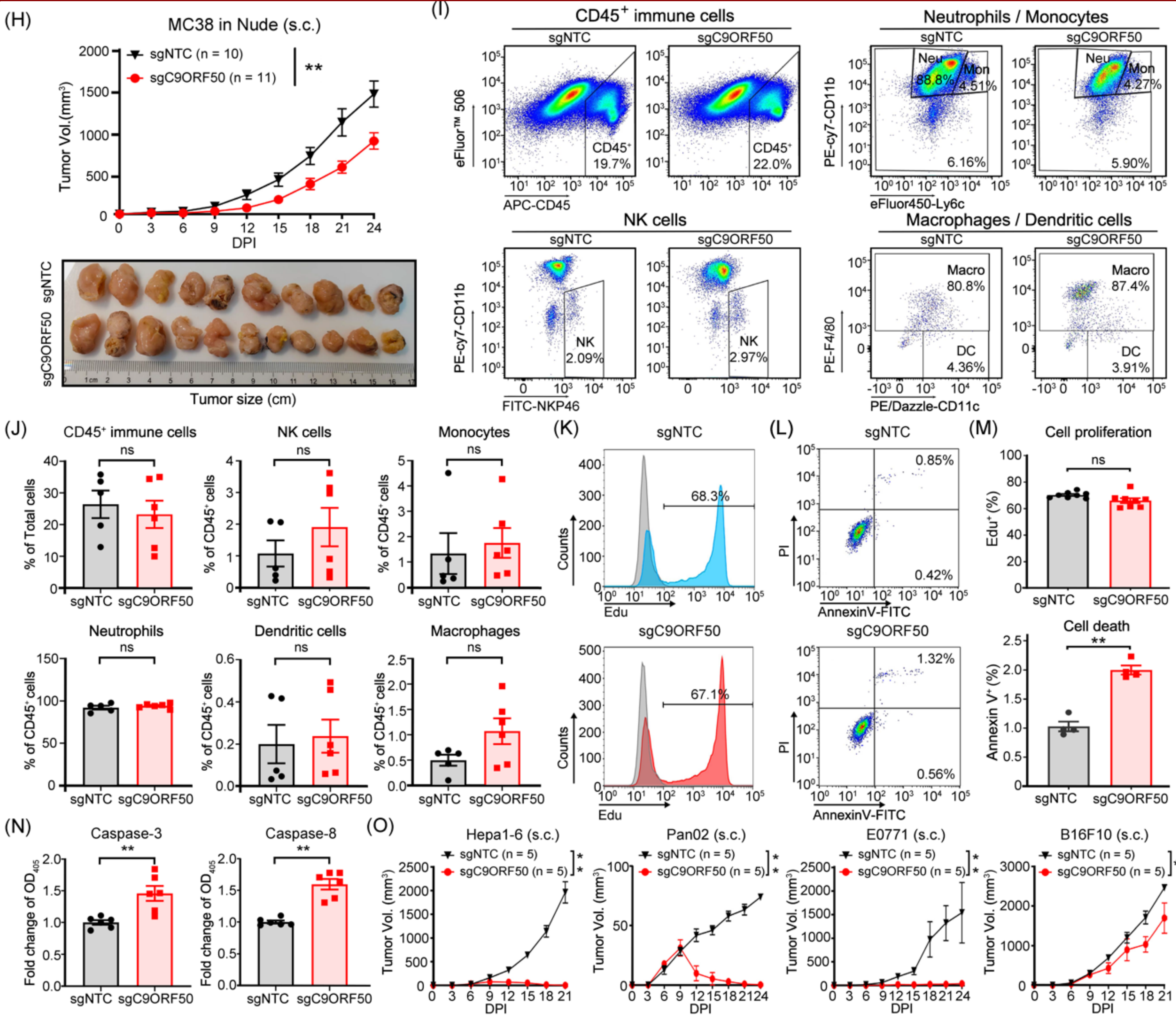


Figure 1 Genome-scale *in vivo* clustered regularly interspaced short palindromic repeats (CRISPR) screen identifies *C9ORF50* as a critical driver of cancer progression.

C9ORF50 is a canonical intrinsically disordered protein driving LLPS

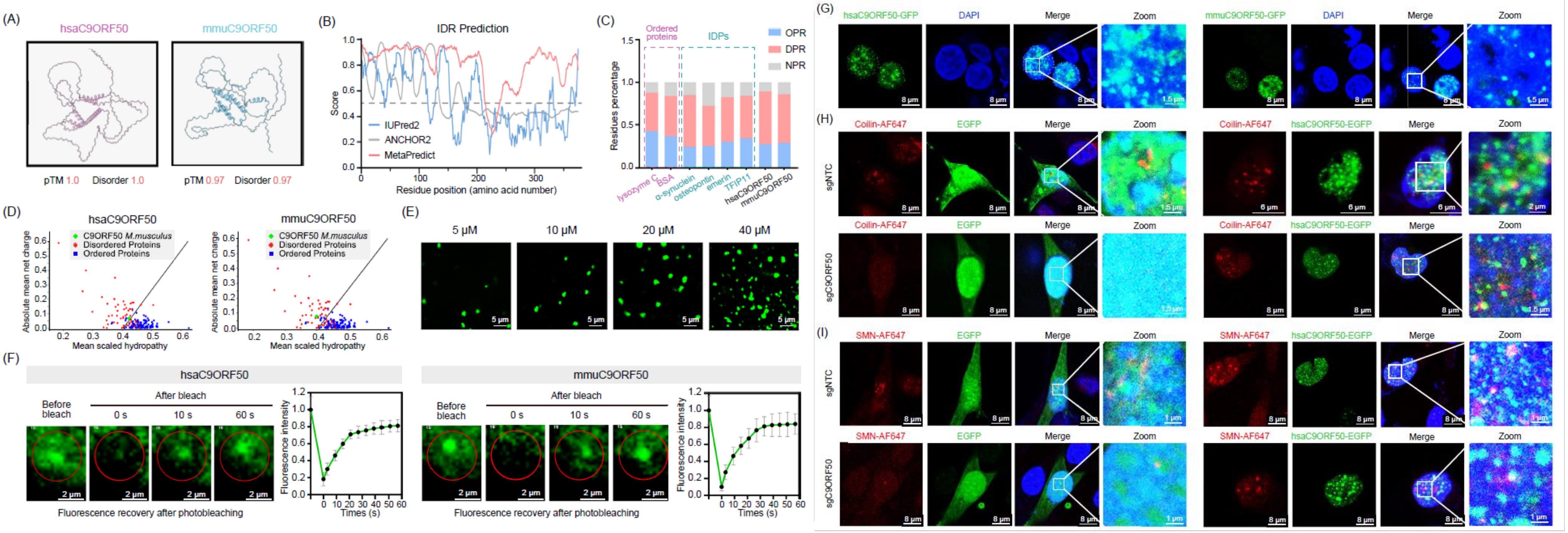


Figure 2 C9ORF50 drives liquid-liquid phase separation (LLPS) to form dynamic nuclear condensates.

C9ORF50 interacts with spliceosome components and influences their expression

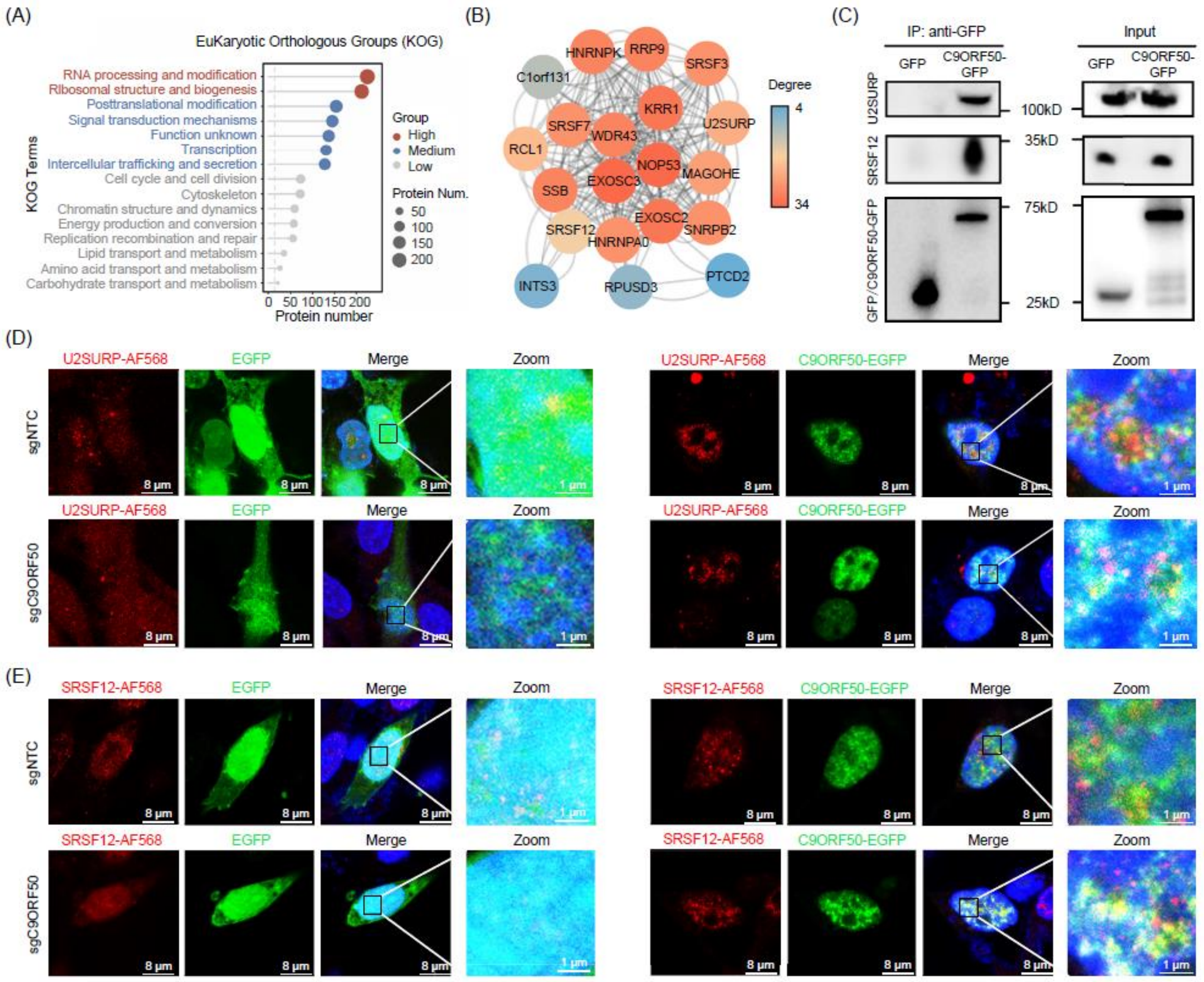
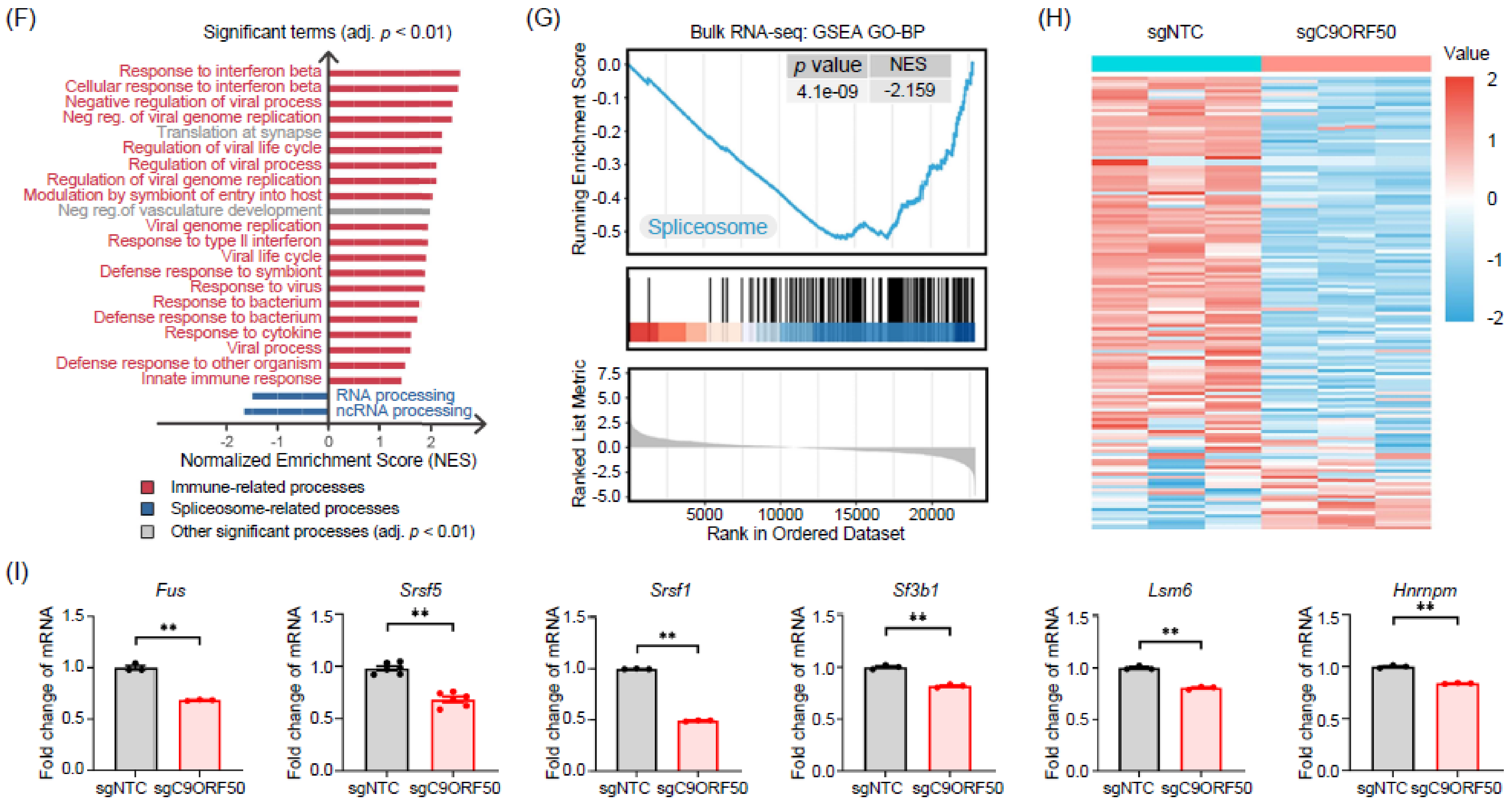


Figure 3 C9ORF50 associates with the spliceosome machinery and regulates spliceosome-related gene expression.

C9ORF50 interacts with spliceosome components and influences their expression



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C9ORF50 deficiency causes intron retention and dsRNA accumulation

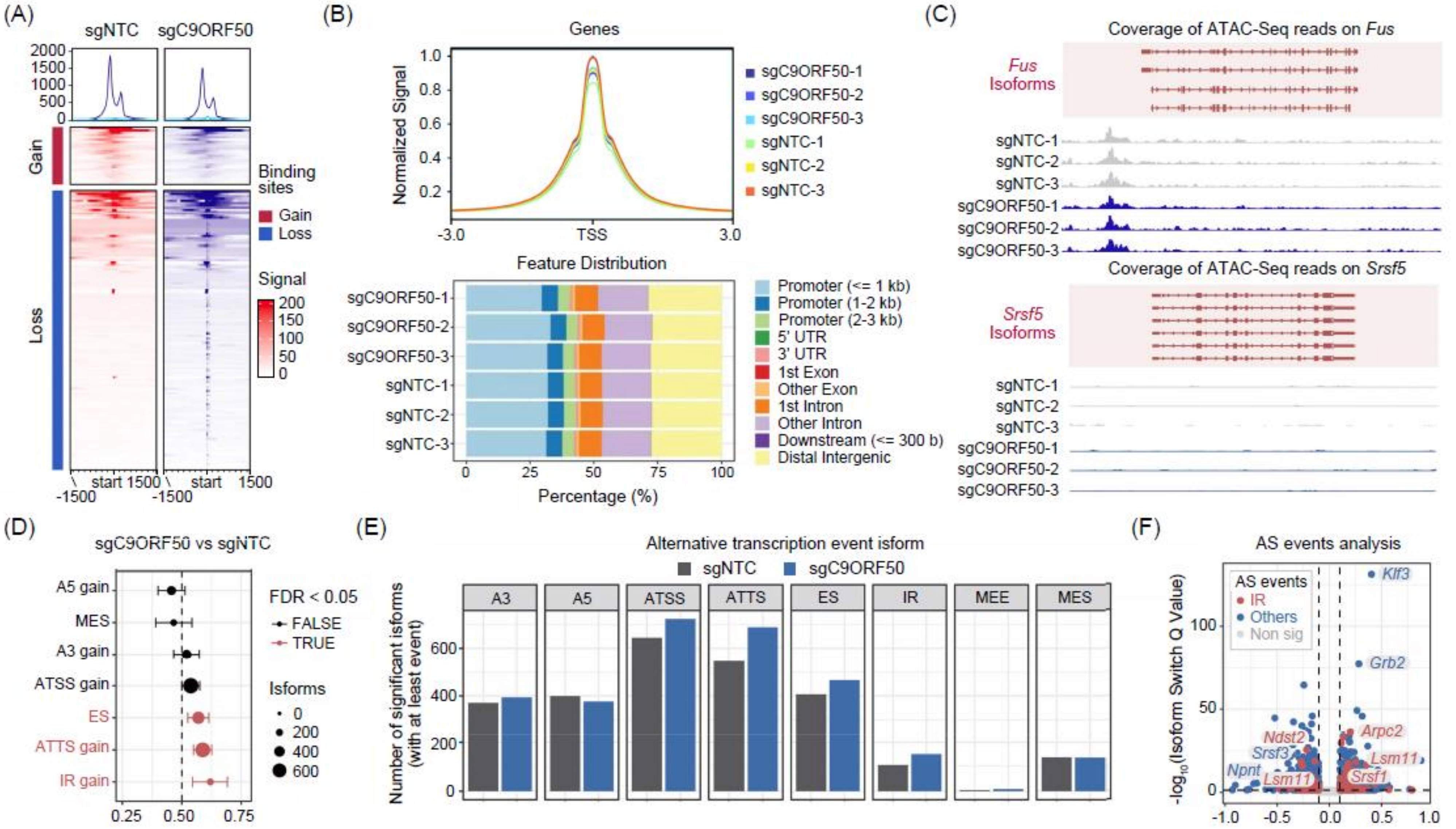

Figure 4 *C9ORF50* deficiency impairs spliceosome function via aberrant alternative splicing events in spliceosome-related genes.

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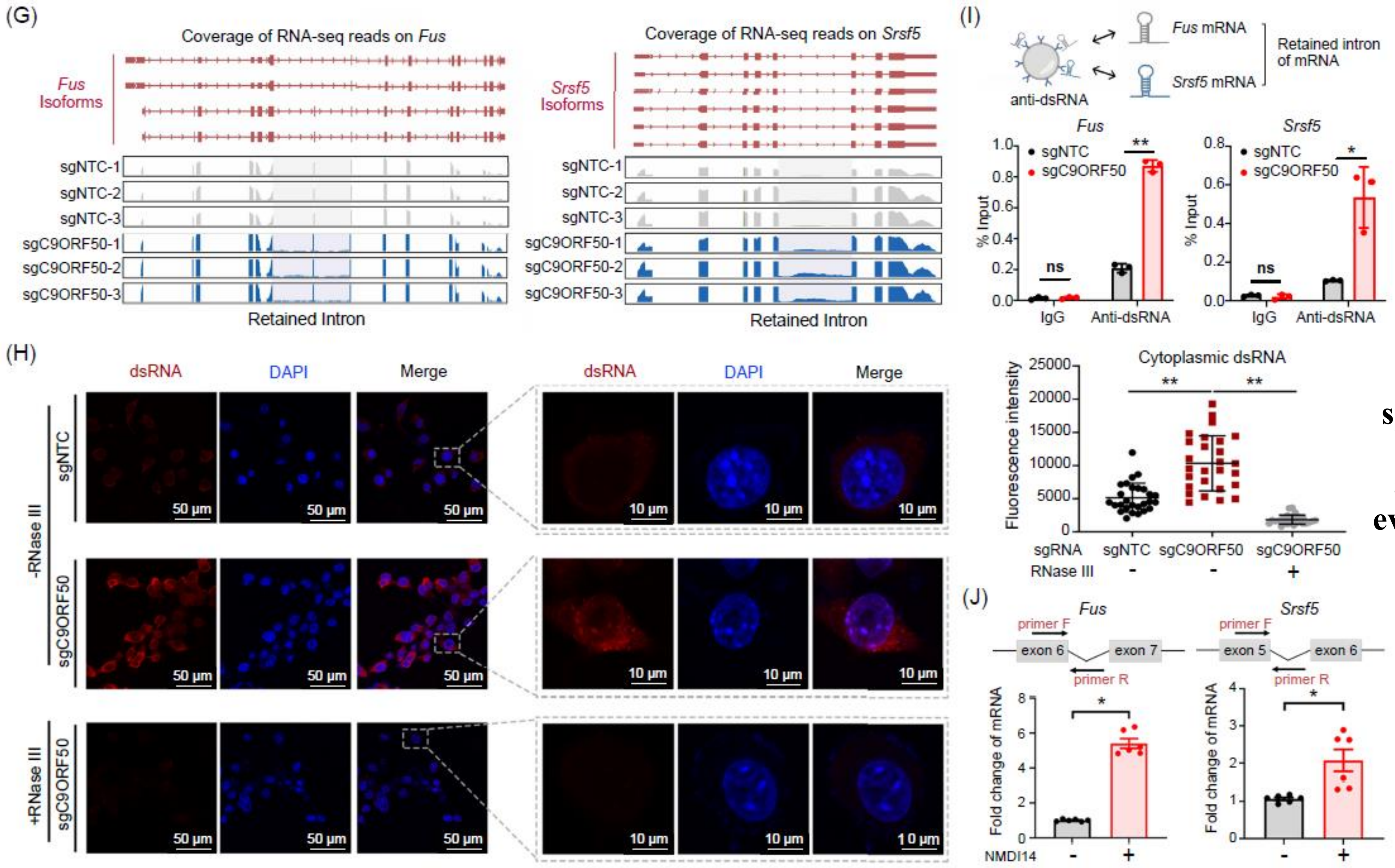

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Cytosolic dsRNA activates innate immune signaling

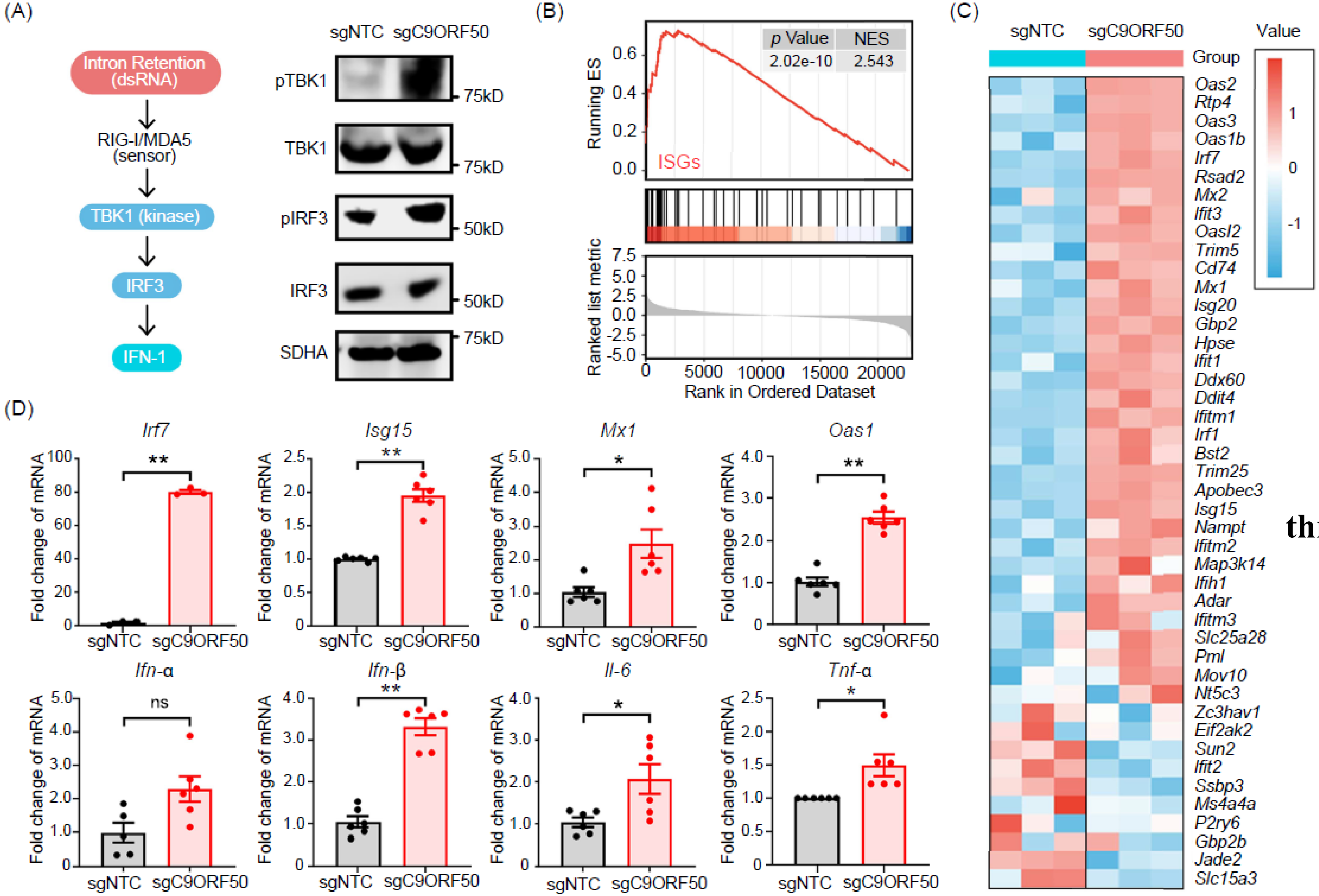

Figure 5 *C9ORF50* knockout activates antitumor immunity through cytoplasmic accumulation of double-stranded RNA.

Cytosolic dsRNA activates innate immune signaling

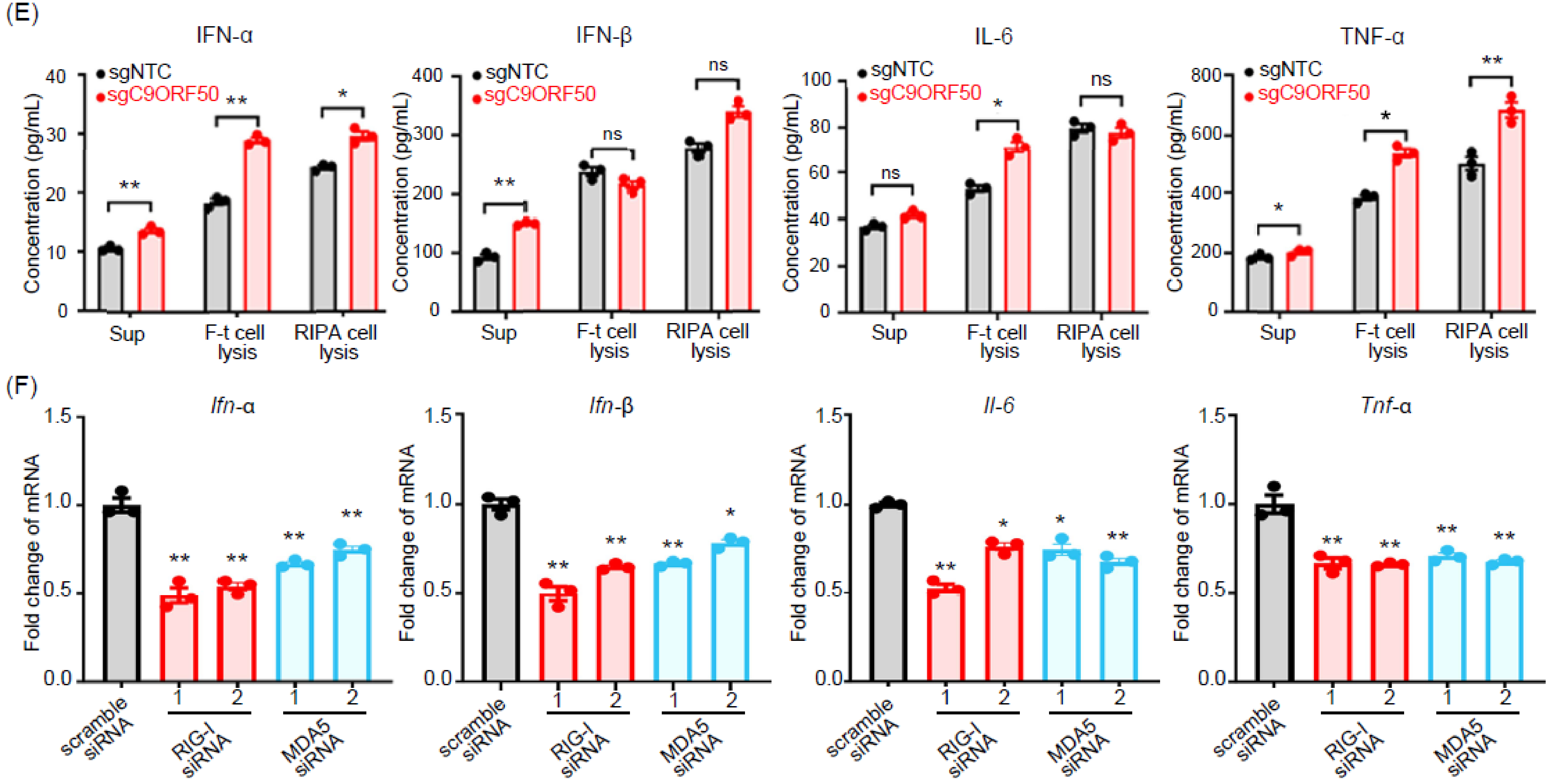

Figure 5 *C9ORF50* knockout activates antitumor immunity through cytoplasmic accumulation of double-stranded RNA.

C9ORF50 depletion remodels the TME with increased T cell infiltration

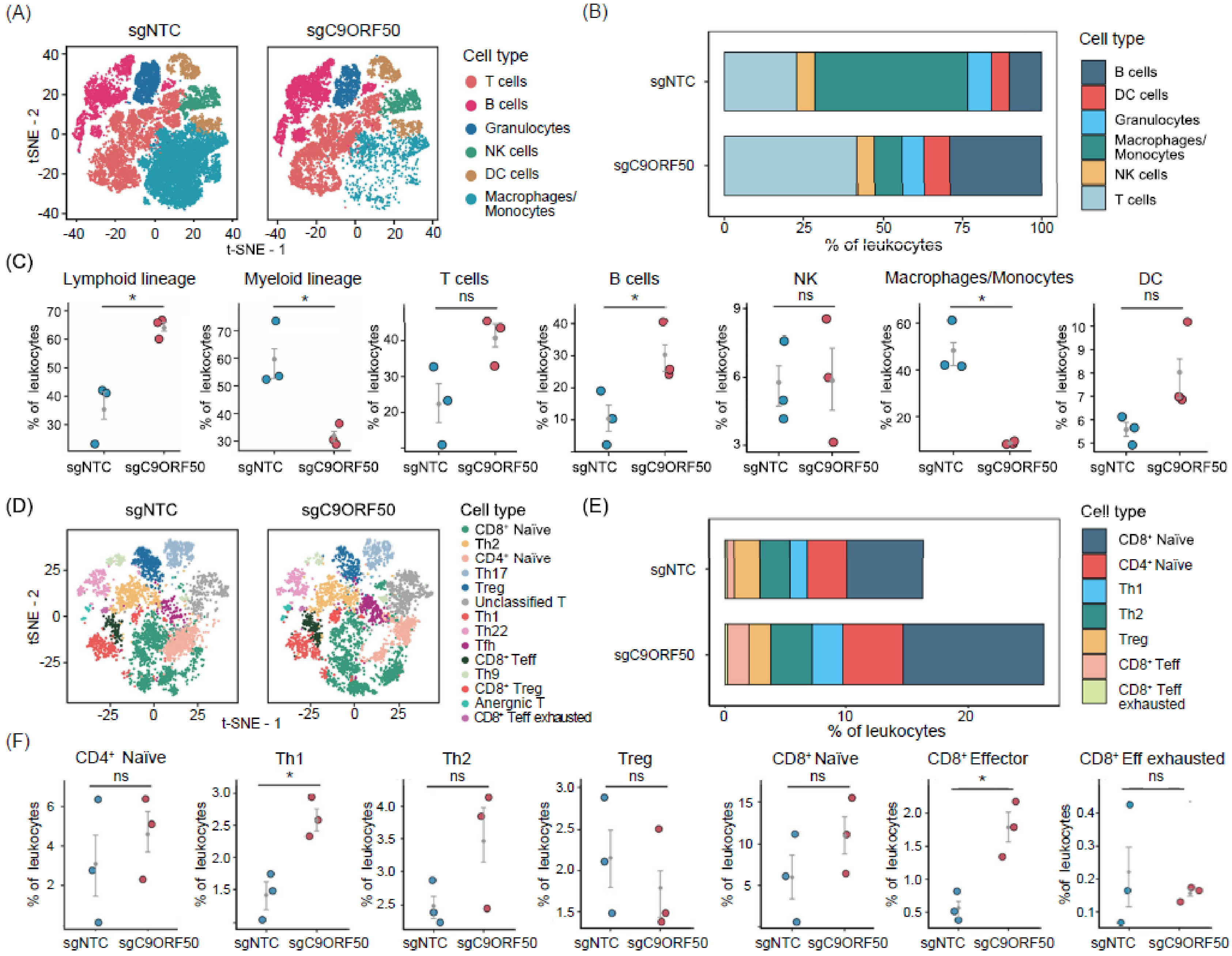

Figure 6 *C9ORF50* knockout remodels the tumor microenvironment (TME) into an immune-stimulatory state.

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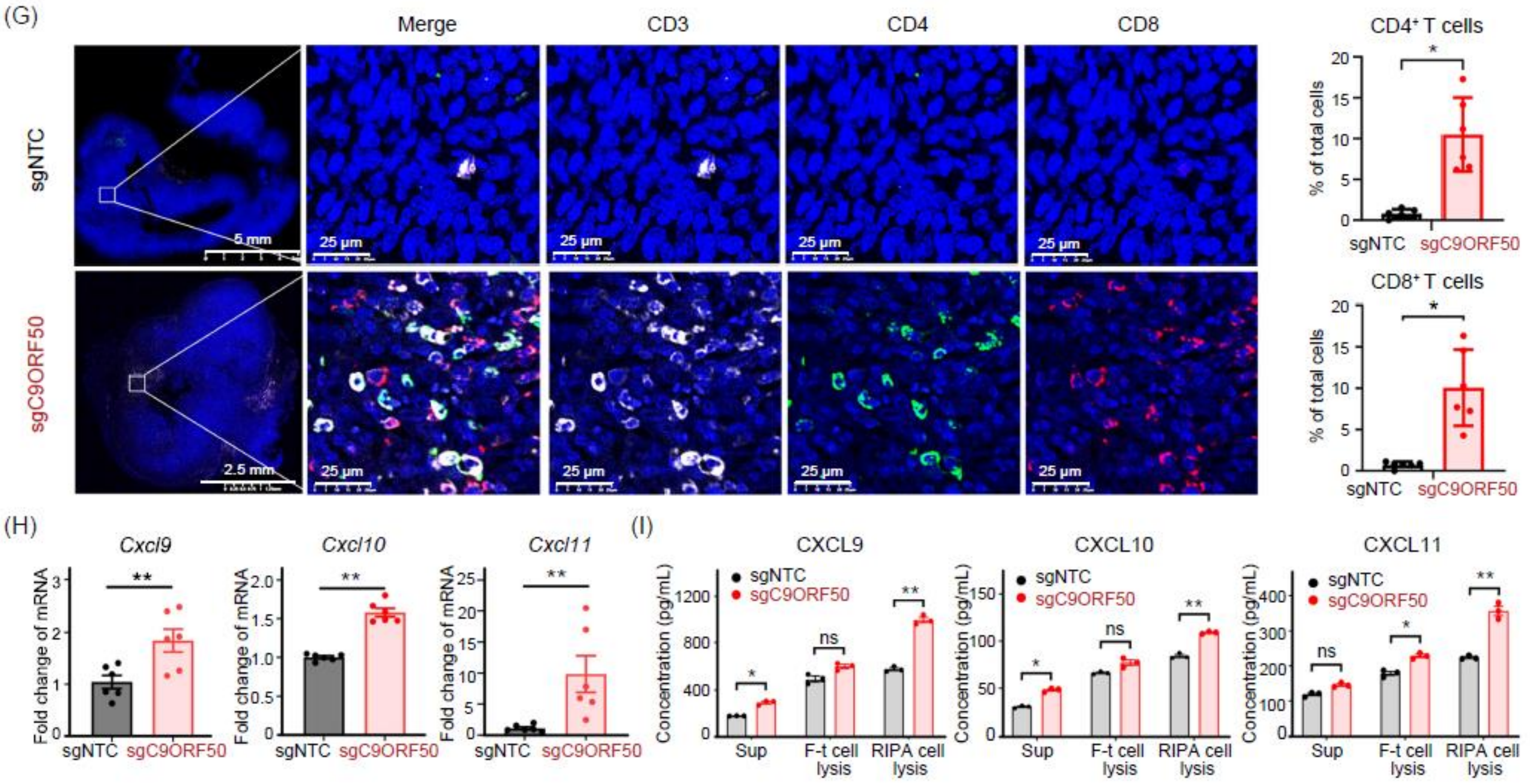

Figure 6 ***C9ORF50*** **knockout remodels the tumor microenvironment (TME) into an immune-stimulatory state.**

Elevated C9ORF50 expression in tumors correlates with poor clinical outcomes

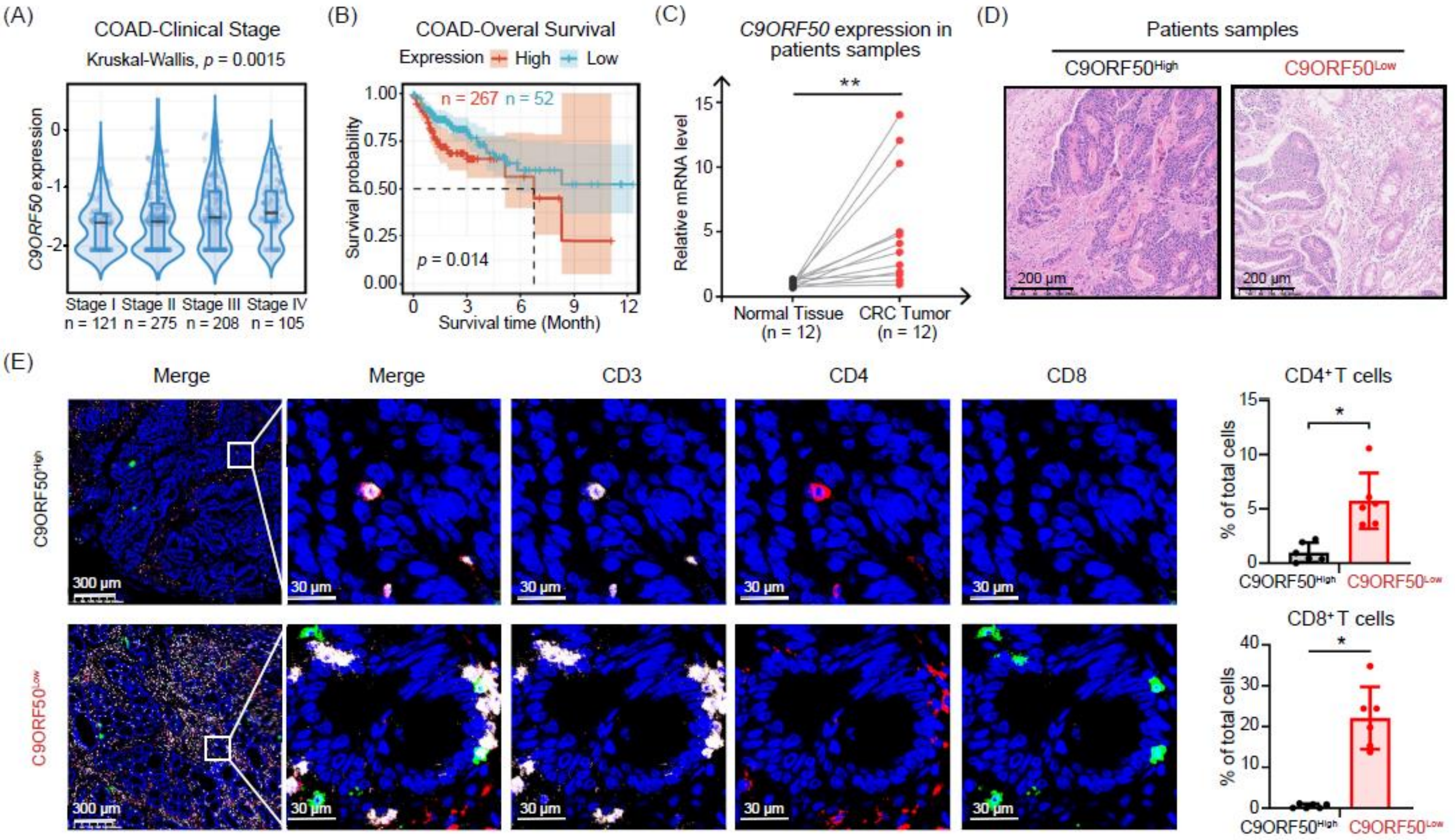

Figure 7 *C9ORF50* correlates with adverse clinical outcomes and exhibits tumor-associated expression.

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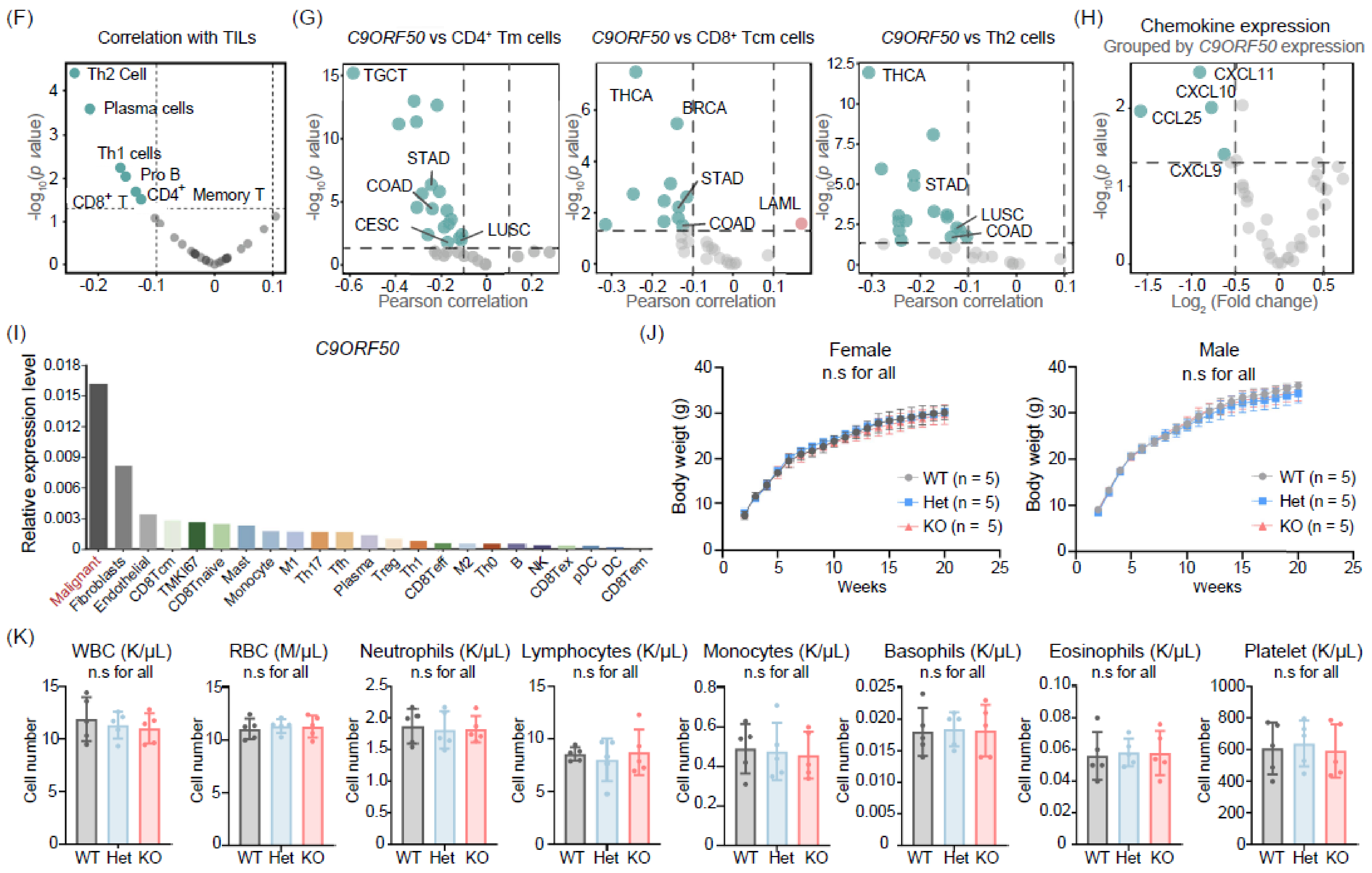

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C9ORF50-targeting RNA interference suppresses cancer growth

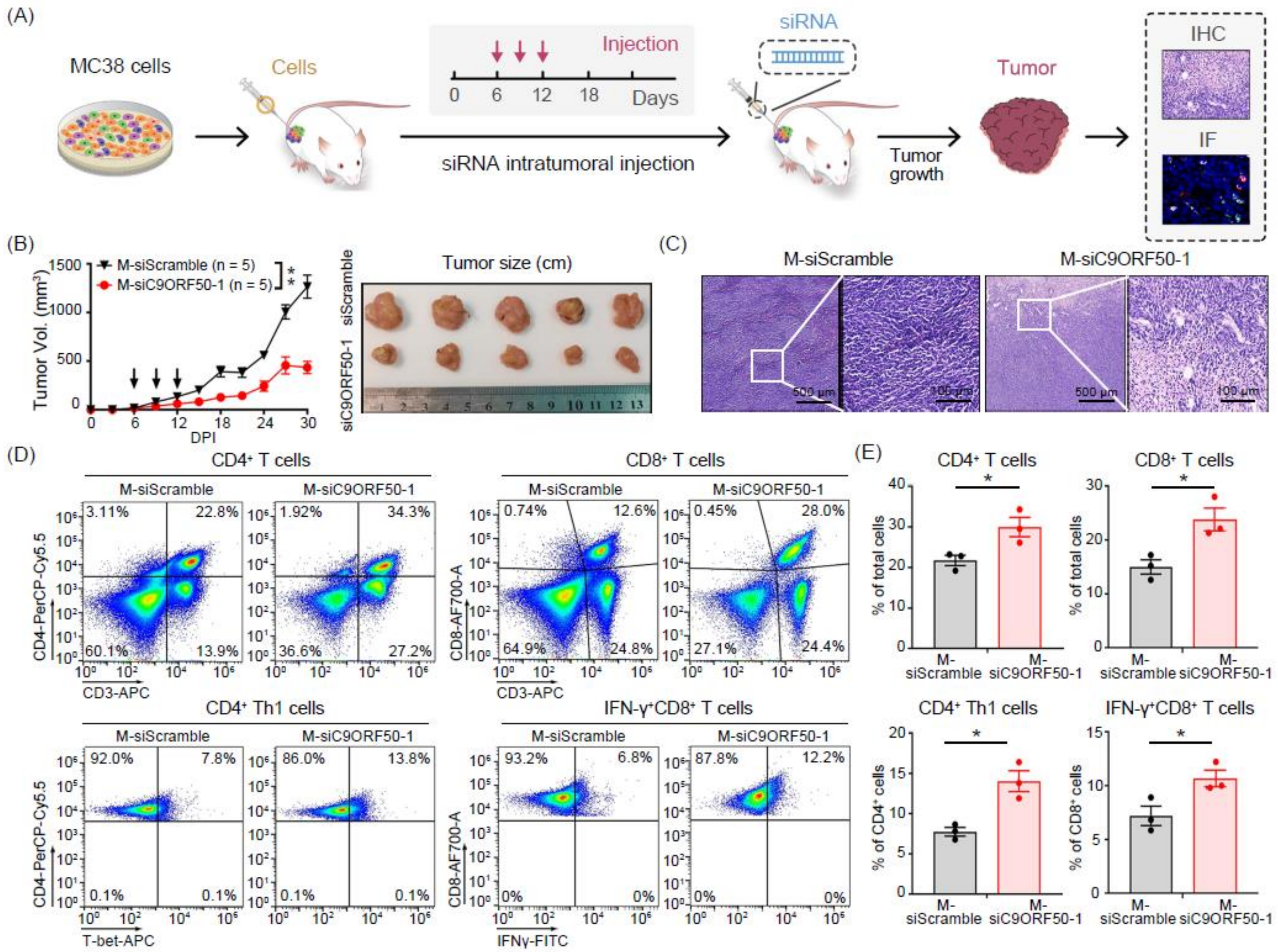

Figure 8 Therapeutic targeting of *C9ORF50* via RNA interference suppresses cancer progression.

C9ORF50-targeting RNA interference suppresses cancer growth

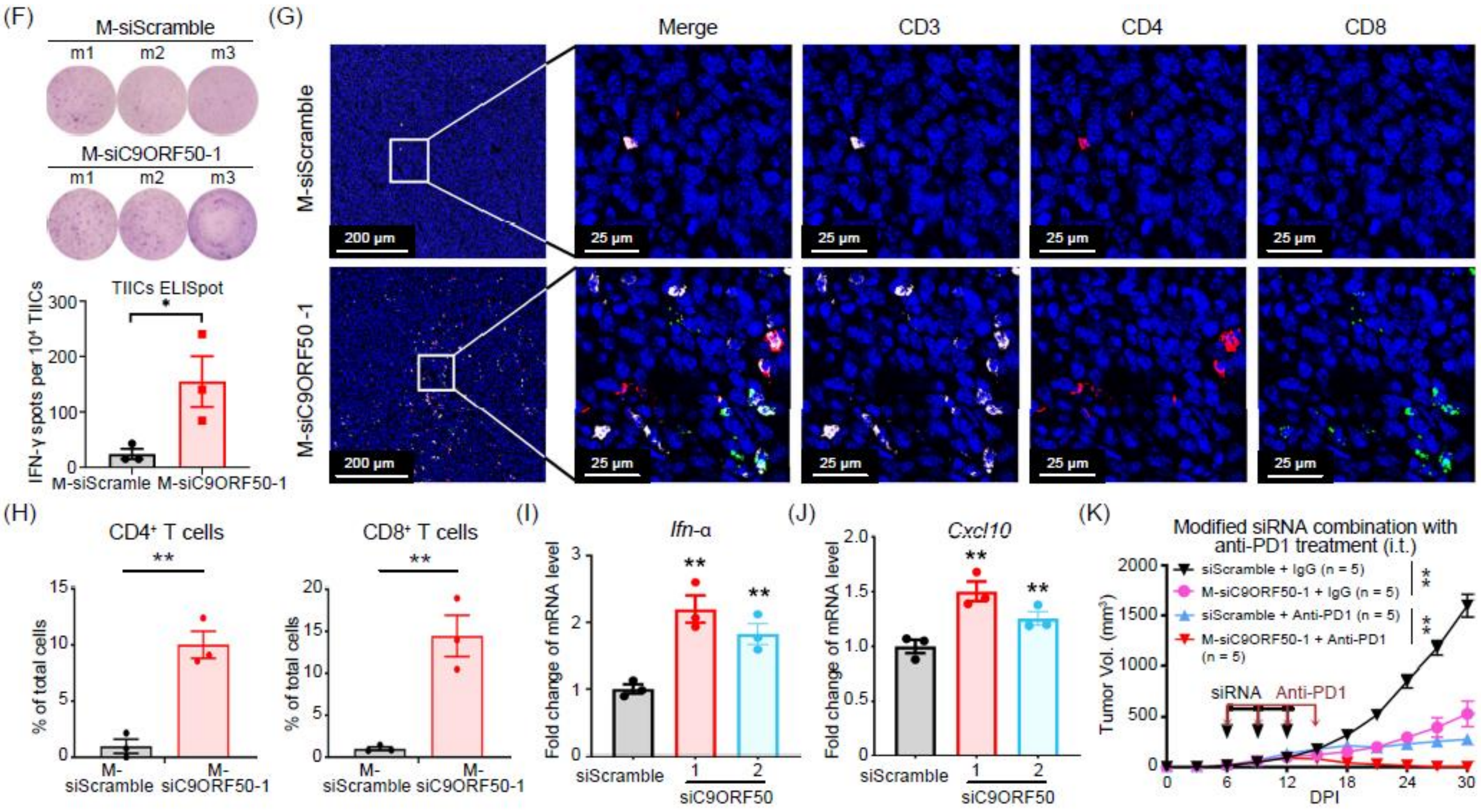

Figure 8 Therapeutic targeting of *C9ORF50* via RNA interference suppresses cancer progression.

Conclusion

- *C9ORF50* is identified as a novel spliceosome-associated protein and a promising target for cancer immunotherapy.

- *C9ORF50* remodels the TME by enhancing immune cell infiltration in poorly infiltrated tumors, thereby increasing their susceptibility to immune-mediated clearance.

- The relatively tumor-enriched expression pattern and non-essential role in normal mouse physiology suggest *C9ORF50* as a promising target for further therapeutic development, though comprehensive safety evaluation will be necessary.

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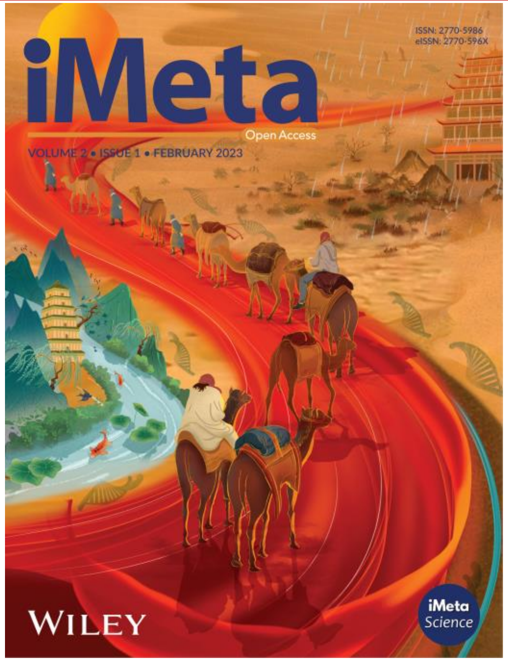

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