



Synthetic promoters for safe and controlled expression in cell and gene therapy

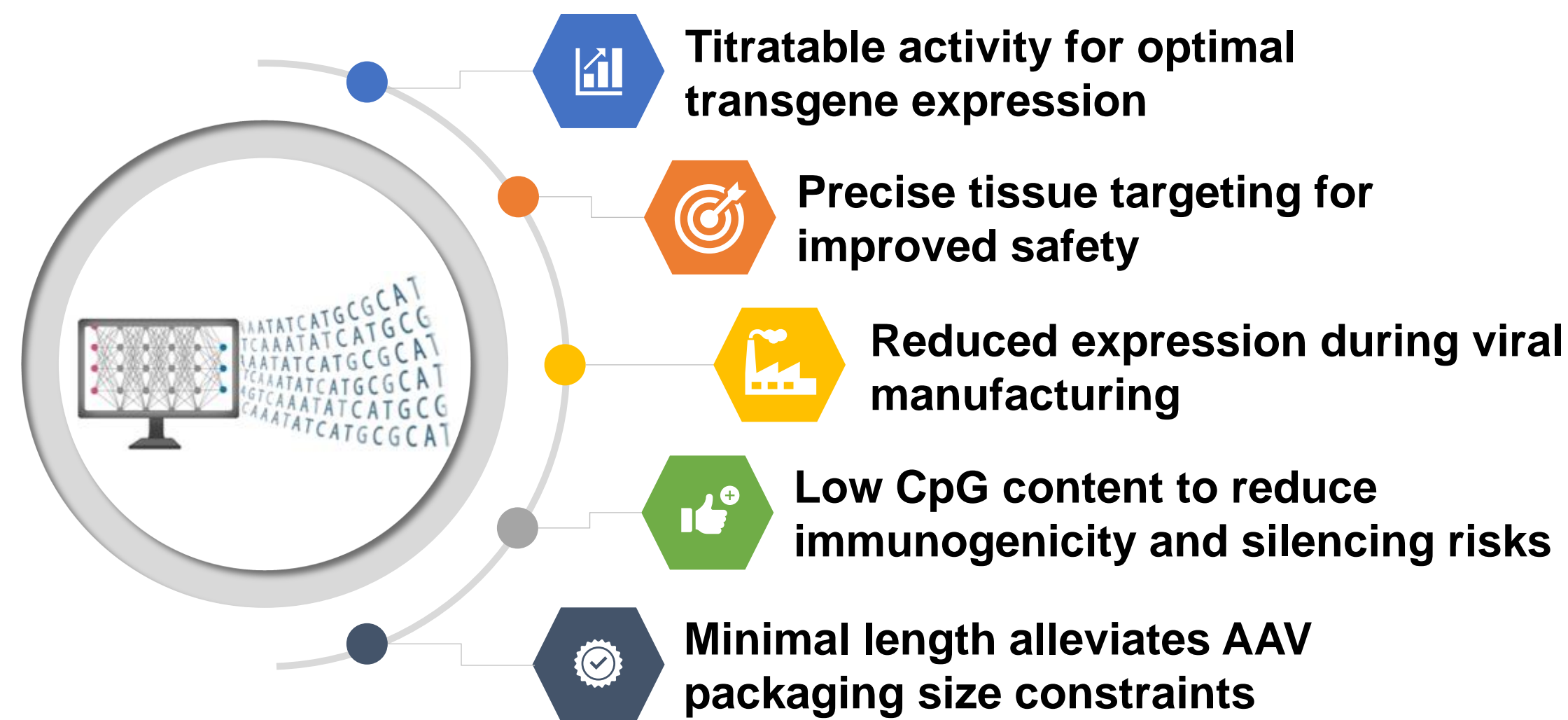
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AO Johnson, F Taglini, MA Smith, P O'Neill, C Alexandru-Crivac, TH Pohle, K Fewkes, AJ Brown, DC James
SynGenSys Ltd, Sheffield, S1 2JE, UK

A computational platform for novel synthetic promoter design



The promoter is a critical component of any gene therapy payload. Determining cell and tissue specificity, transgene expression levels and expression stability. Its selection directly impacts safety and efficacy. Traditional viral and native promoters are suboptimal for many intended applications. Instead, synthetic promoters enable precise control to maximize therapeutic benefits, minimize off-target effects, reduce dosing, and prevent transgene expression during manufacturing.

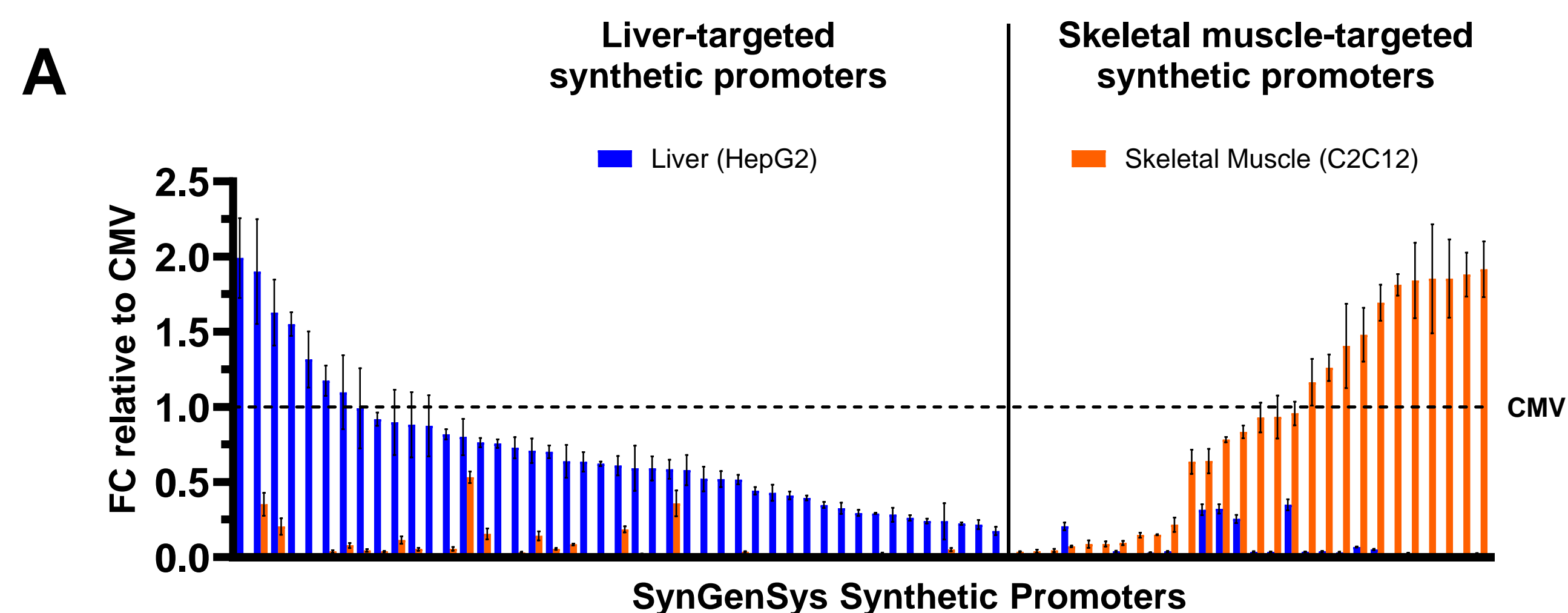
Our proprietary computational platform rapidly surveys the transcriptional landscapes of all human cell and tissue types to mine subsets of transcriptionally active DNA sequences. These are then assembled *in silico* into synthetic promoters according to user-defined requirements, such as tissue specificity, activity, and low immunogenicity.

We exemplify here the capability of our platform to design novel, patentable liver-, muscle-, and NK-targeted synthetic promoters showing a broad range of activity in the target cells and negligible activity in off-target cells.

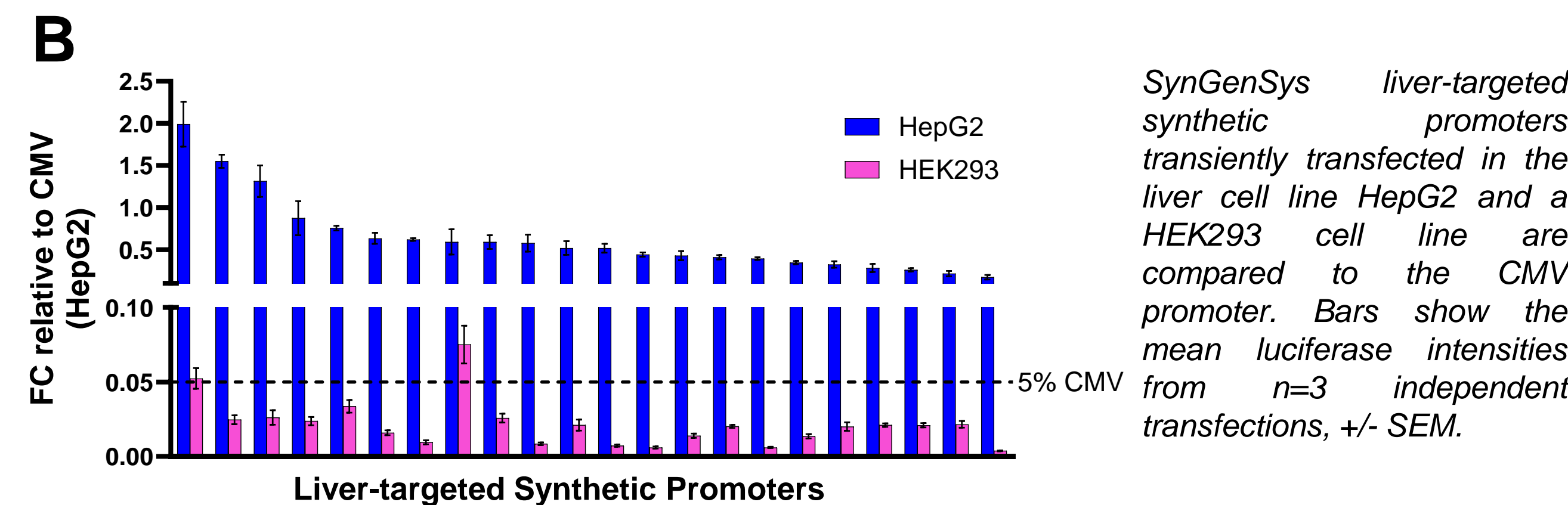
Titratable Expression with Liver-targeted Synthetic Promoters

SynGenSys demonstrates control over liver and skeletal muscle targeted expression with a library of patentable off-the-shelf synthetic promoters characterised by:

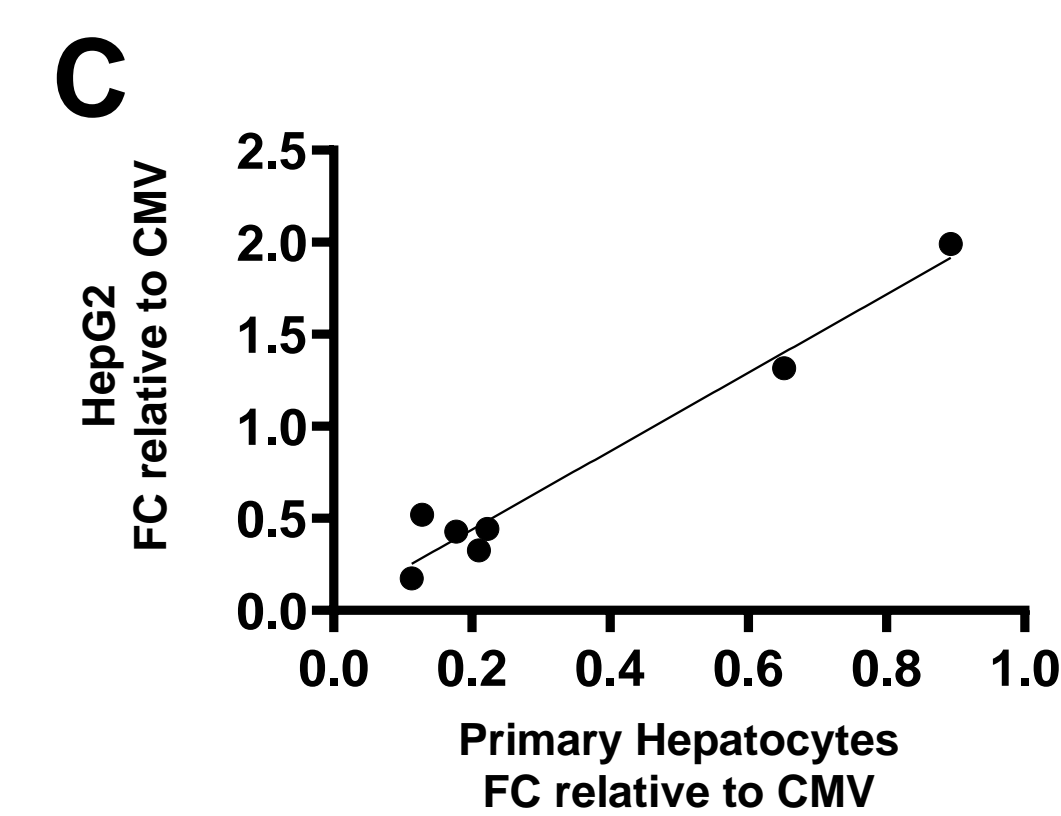
- ✓ Over 10-fold range of expression in the intended target cell line with minimal activity in an off-target cell line (A)
- ✓ Reduced expression in HEK293 producer cells for improved AAV manufacturing (B)
- ✓ Reproducibility of expression between the HepG2 cell line and primary human hepatocytes (C), and plasmid DNA and AAVs (D)
- ✓ Smaller size and fewer CpGs compared to the hCMV promoter



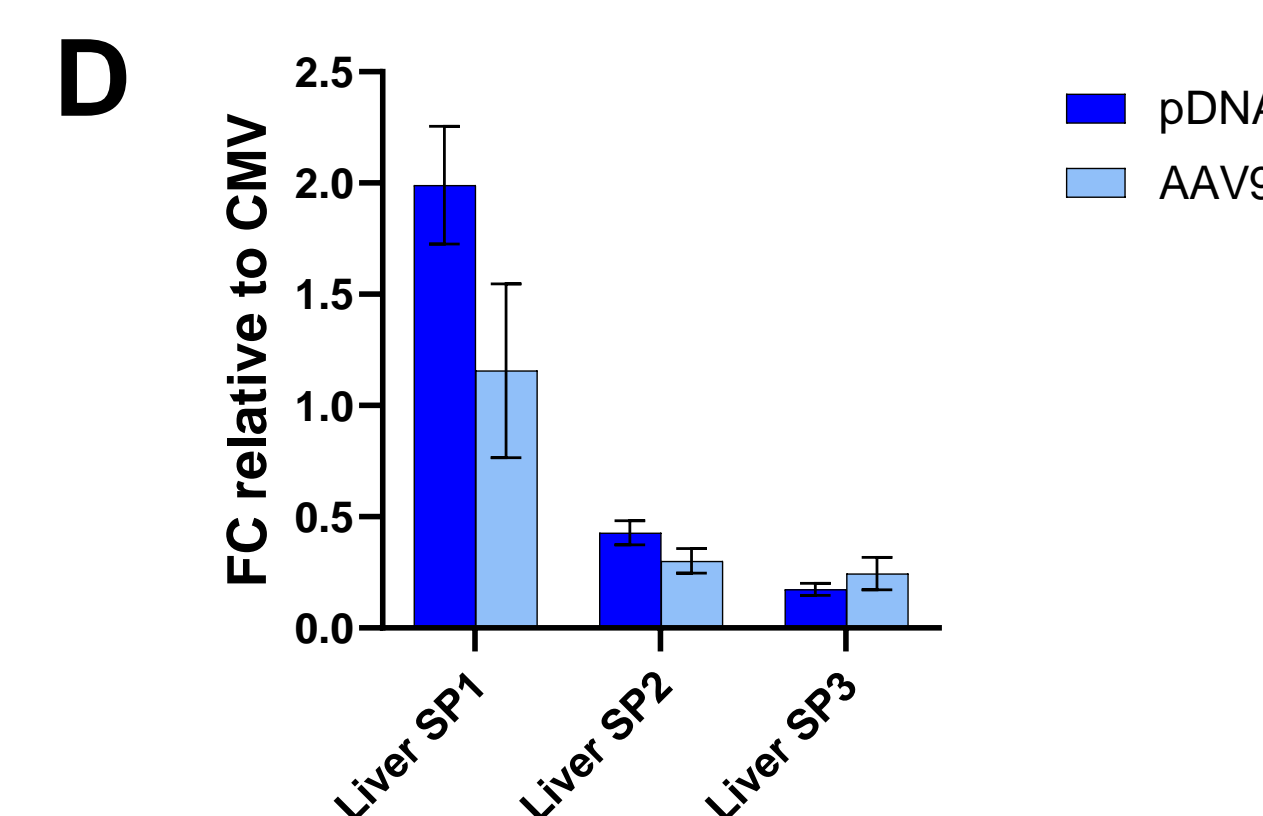
SynGenSys liver and skeletal muscle targeted synthetic promoters transiently transfected in the liver cell line HepG2 and the skeletal muscle cell line C2C12 are compared to the CMV promoter. Bars show the mean luciferase intensities from n=3 independent transfections, +/- SEM.



SynGenSys liver-targeted synthetic promoters transiently transfected in the liver cell line HepG2 and a HEK293 cell line are compared to the CMV promoter. Bars show the mean luciferase intensities from n=3 independent transfections, +/- SEM.



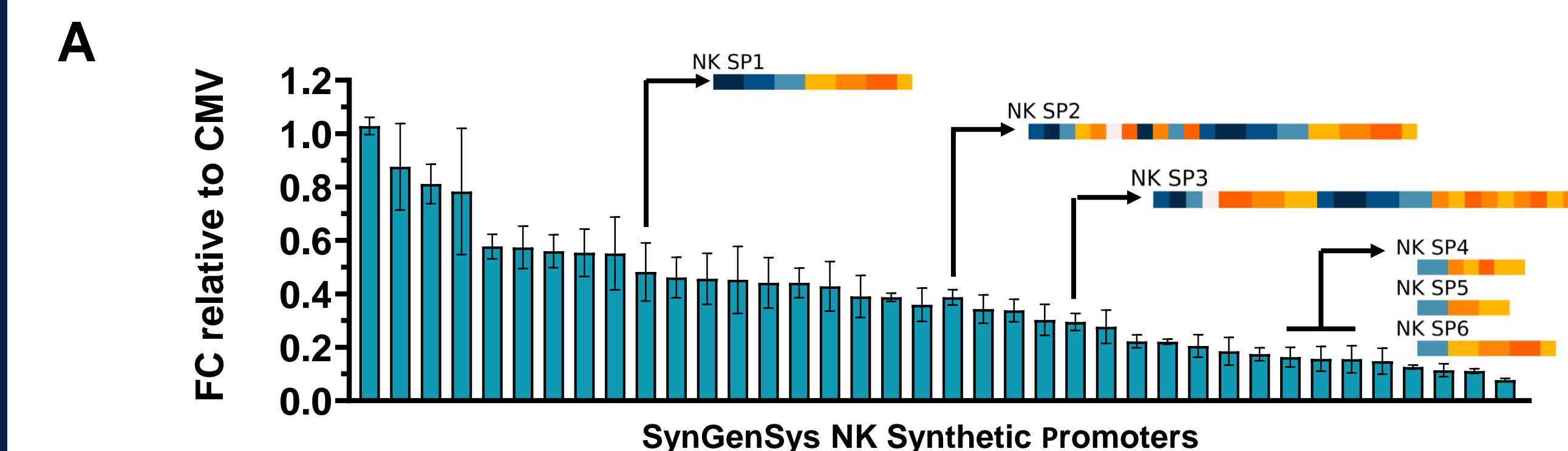
Correlation between the mean promoter activity in HepG2 cells and primary human hepatocytes for a selection of liver-targeted SynGenSys promoters. n=3 independent transfections, and primary human hepatocytes from 3 different donors (C). The HepG2 cell line was transfected with plasmid DNA or transduced with AAV9 vectors encoding luciferase under the control of three selected SynGenSys liver-targeted synthetic promoters. Bars show the mean luciferase intensities from n=3 independent transfections, +/- SEM (D).



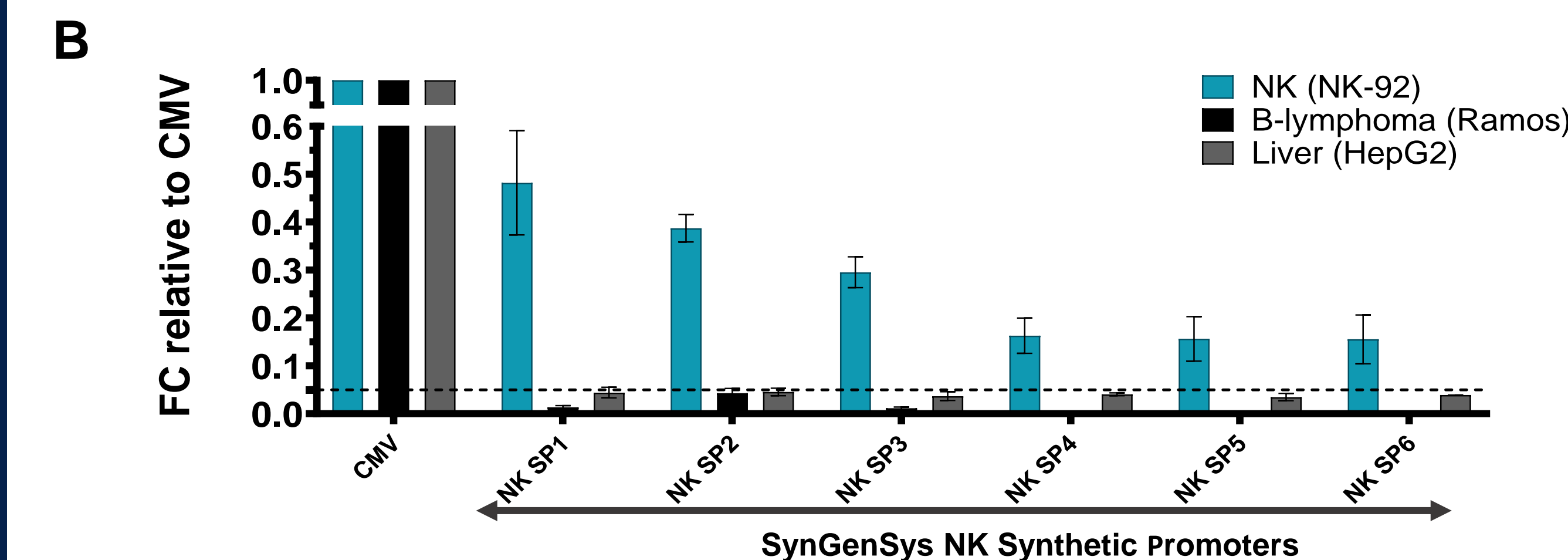
Highly Targeted NK Promoters

SynGenSys has developed a library of novel off-the-shelf NK synthetic promoters that exhibit:

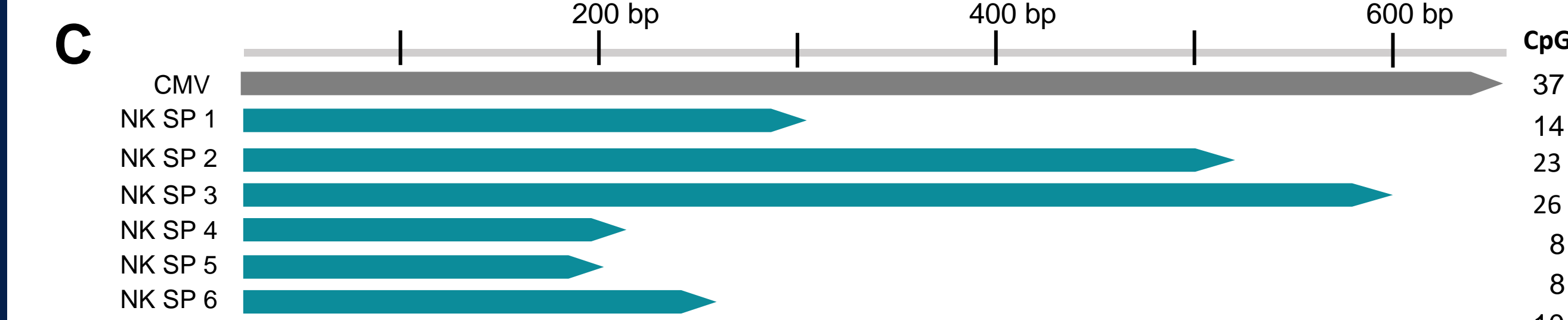
- ✓ A broad range of activity in target NK-92 cells (A)
- ✓ Pre-designed minimal off-target expression in Ramos B-cell lymphoma and HepG2 hepatocyte cells (B)
- ✓ Minimal size (590-208 bp)
- ✓ Lower CpG content than CMV (8-26 CpGs vs 37 CpGs in the CMV promoter)



SynGenSys NK synthetic promoters transiently transfected in NK-92 cells are compared to the CMV promoter. Bars show the mean GFP intensities from at least n=3 independent transfections, +/- SEM.



SynGenSys NK-targeted synthetic promoters transiently transfected in NK-92 cells, Ramos and HepG2 cells are compared to the CMV promoter. Bars show the mean GFP intensities from at least n=3 independent transfections, +/- SEM. Dotted line at 5% activity relative to CMV promoter.



Sizes and CpG content of selected SynGenSys NK-targeted synthetic promoters.