

**A sensitive, non-radioactive assay for Zn(II) uptake into metazoan cells**

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## I. Chemicals and Instrumentation

A. Chemicals and consumables: Phosphate buffered saline was purchased either as a 1× solution 21-040-CV) or a 10x concentrate (46-013-CM) from Corning. Tris (tris(hydroxymethyl)aminomethane) was purchased as a solid from Millipore Sigma (T1503, ≥ 99.5%). HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) was purchased as a solid from Millipore Sigma (either RDD002 or H3375, both ≥ 99.5%). Sodium chloride was purchased as a solid from Millipore Sigma (S7653, ≥ 99.5%). Ammonium sulfate was purchased as a solid from Mallinckrodt (3512). Sodium hydroxide was purchased as a solid from Mallinckrodt (7708-06). Triton X-100 was purchased from Alfa Aesar (A16046). PMSF was purchased as a solid from Amresco (M145). TPEN (*N,N,N',N'*-tetrakis(2-pyridylmethyl)ethylenediamine) was purchased from Millipore Sigma (P4413-100MG) as a solid and dissolved in DMSO to a final concentration of 20 mM. Dulbecco's Modification of Eagle's Medium (DMEM) (15-017-CM) was purchased from Corning as a solution to which glutamine (Corning, 25-005-CI, 2 mM final concentration) and penicillin-streptomycin (Corning, 30-002-CI, 100 IU/L penicillin and 100 µg/mL streptomycin final concentration) were added. Fetal Bovine Serum (FBS) was purchased from Corning (35-010-CV). Nitric acid was purchased from Aristar Ultra as a concentrated solution (87003-226, 70%, ultra-high purity for quantitative trace metal analysis at the part per trillion level). ICP-MS environmental calibration standard was purchased from Agilent as a solution in 10% nitric acid (5183-4688). Amicon Ultra-15 molecular weight cutoff filters were purchased from Millipore (UFC901008). Pierce™ NHS-Activated Agarose, dry, was purchased from VWR (PI26196). <sup>70</sup>Zn(II)-gluconate was purchased as a solid from Millipore-Sigma (702595-CONF). Agilent ICP-MS Tb internal standard was purchased from Agilent (5190-8590, Tb as Tb<sub>4</sub>O<sub>7</sub> at 10,000 ppb in 2% HNO). ICP-MS Sample Tubes were purchased from VWR (97012-778). Sodium pyrithione was obtained as a solid from Sigma. Aqueous stock solutions were prepared from high-purity salts: CdCl<sub>2</sub> (99.999%; Aldrich), CoCl<sub>2</sub> (99.9%; Strem), CuCl<sub>2</sub>·2H<sub>2</sub>O (99.999+%; Aldrich), MnCl<sub>2</sub> (99.99% metals basis; Alfa Aesar), NiCl<sub>2</sub>·6H<sub>2</sub>O

(99.9999%; Aldrich), and  $\text{ZnCl}_2$  (99.99% metals basis; Alfa Aesar). pENTR233 vectors encoding ZIP4 (HsCD00370180), ZIP8 (HsCd00372914), and ZIP10 (HsCd00399932) were purchased from the PlasmidID Repository at Harvard Medical School. Vector sequences were confirmed by Sanger sequencing.

- B. Instrumentation: ICP-MS was performed on an Agilent 7900 ICP-MS in helium mode equipped with an integrated autosampler at the MIT CEHS core facility. The internal standard Tb was monitored.

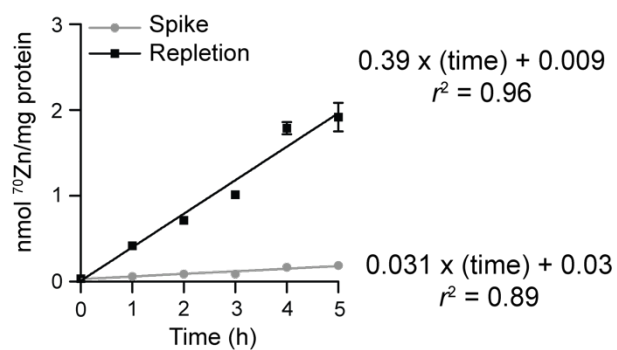
**Table S1.** Primers used for gene amplification and qPCR.

Gene	Sense Primer	Antisense Primer
ZIP4	5'- AAAAAAGGTACCAA ATGGCGTCCCTGGT -3'	5'- AAAAAATCTAGATTAGAAGG TGATGTCATCCTCGTA -3'
ZIP8	5'- AAAAAAGGTACCA AATGGCCCCGGT -3'	5'- AAAAAACTCGAGTTACTC CAATTCGATTTCTCCTGC -3'
ZIP10	5'- AAAAAAGGTACCATGAAGG TACATATGCACACAAAAT -3'	5'- AAAAAACTCGAGTTAAAACT GGATGTCAAACACAATTTTATC -3'
qPCR RPLP2	5'-CCATTCAGTCACTGATAACCTTG-3'	5'-CGTCGCCTCCTACCTGCT-3'
qPCR MT1A	5'-TGGACCCCACTGCTCCTG-3'	5'-CTTCTCTGATGCCCTTTGC-3'
qPCR ZIP4	5'-GTGTTCTGCCACGAGTTGCC-3'	5'-CTCGCTCTCCTCGCTGACTC-3'
qPCR ZIP8	5'-GCAAGGCTTGTCGAGTGCTC-3'	5'-ATTCATCGATGGCCTGGCG-3'
qPCR ZIP10	5'-GGCCAACAAGAATCCCCTCCT-3'	5'-CCGTGGTGGTTATGTGCAGC-3'

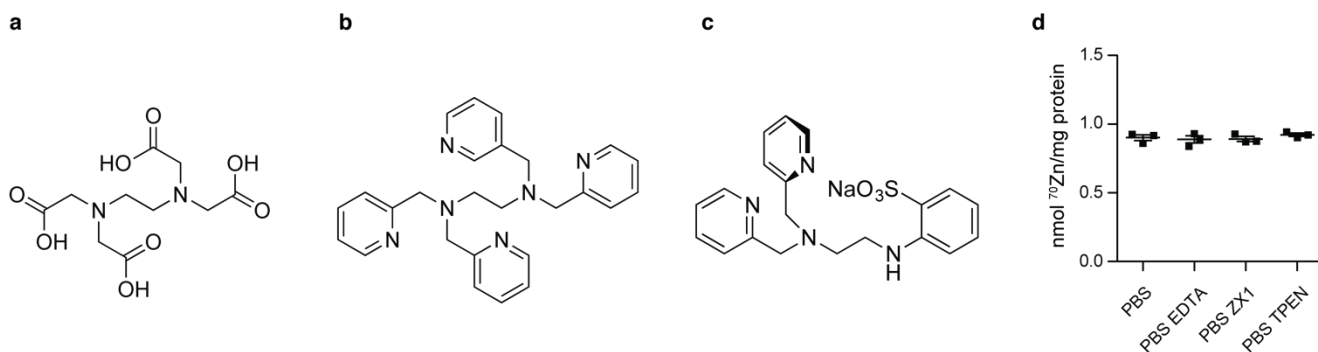
**Table S2.** Metal and Zn isotope concentrations (ppb) in a typical preparation of <sup>70</sup>Zn(II)-repleted DMEM/FBS. N.D indicates not detected.

Metal Concentration / ppb												
Mg	Ca	Mn	Fe	Co	Ni	Cu	<sup>64</sup> Zn	<sup>66</sup> Zn	<sup>67</sup> Zn	<sup>68</sup> Zn	<sup>70</sup> Zn	Cd
19600	68000	40	200	0.2	3	14	20	26	5	23	170	N.D.

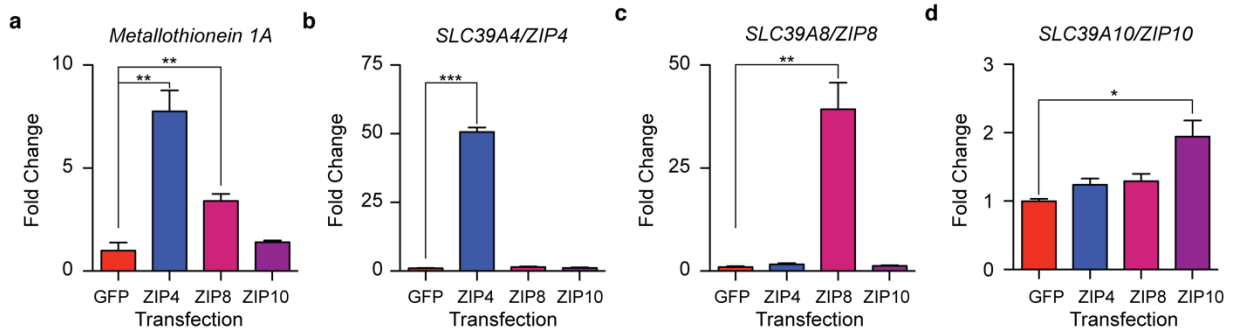
## Supporting Figures:



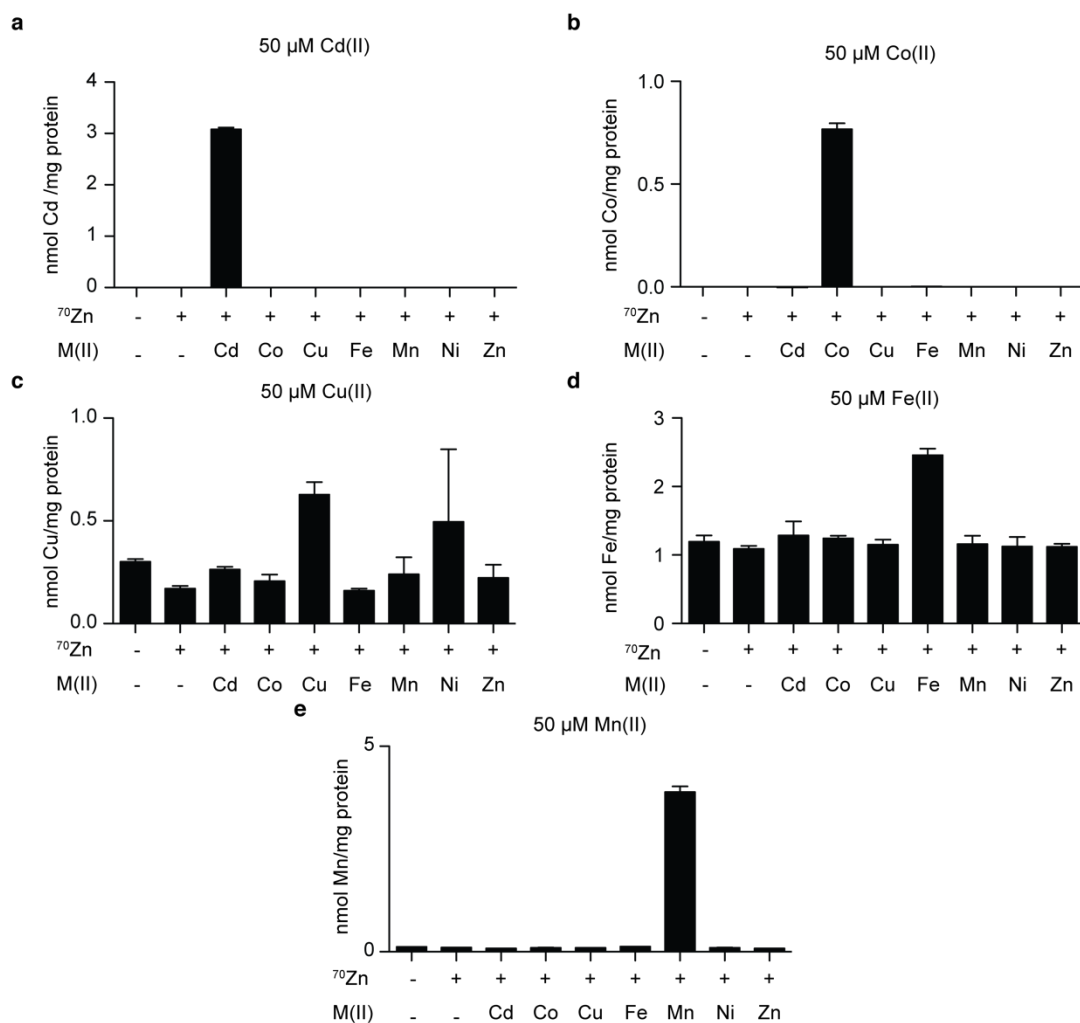
**Figure S1.** Comparison of  $^{70}\text{Zn}(\text{II})$  uptake from HEK293T cells cultured in DMEM/FBS spiked with 20 ppb  $^{70}\text{Zn}(\text{II})$  versus medium depleted of natural abundance Zn(II) then repleted with  $^{70}\text{Zn}(\text{II})$  (N = 3,  $\pm$ SEM). The latter protocol affords a > 10-fold improved signal.



**Figure S2.** Structures of (a) EDTA, (b) TPEN, and (c) ZX1. (d) Cells washed with PBS containing 100  $\mu$ M of these chelators before harvesting do not have significantly different concentrations of cell-associated zinc ( $N = 3$ ,  $\pm$ SEM).



**Figure S3.** (a–d) qPCR analysis for transcripts for metallothionein 1A and ZIP family transporters (N = 3,  $\pm$ SEM). The gene name and protein name corresponding to the transcript analyzed in each panel is indicated above each plot.

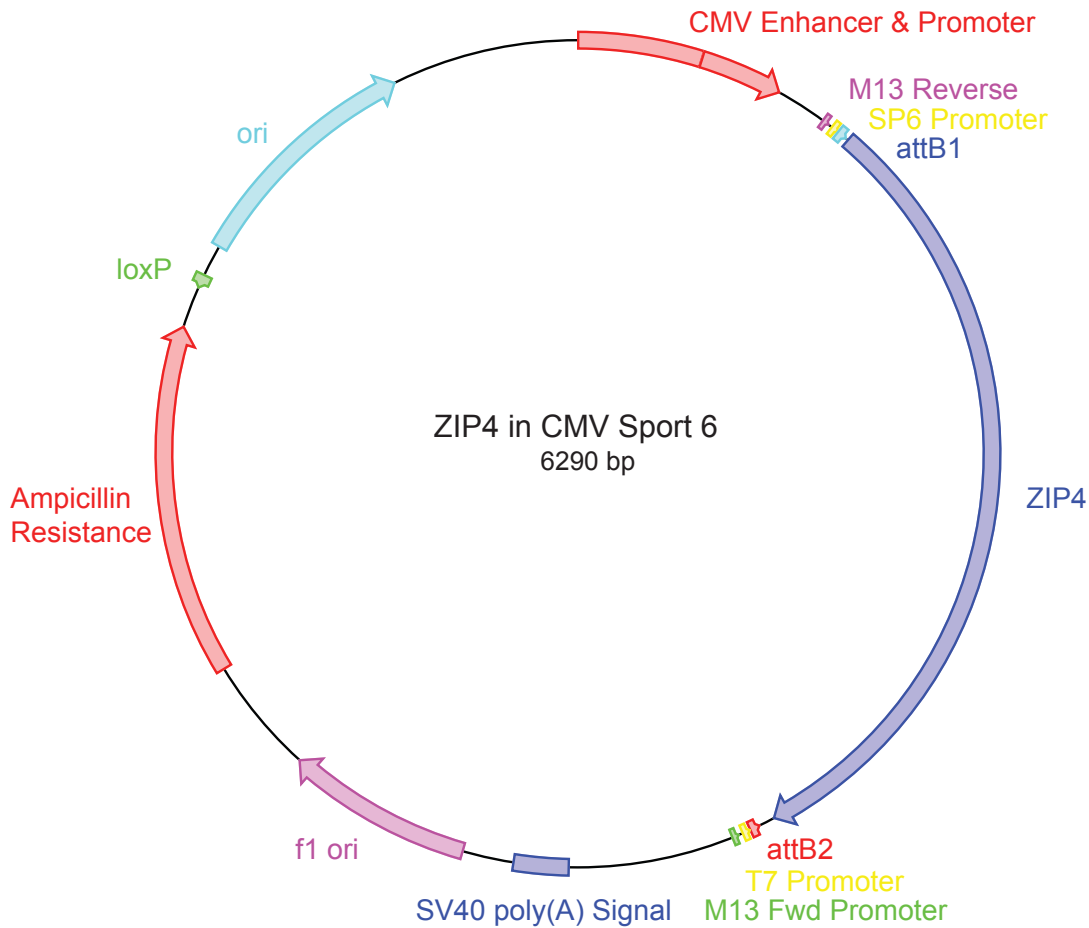


**Figure S4.** (a–e) Normalized cellular metal concentrations from HEK293T cells stimulated with 50  $\mu\text{M}$  of the indicated metal. Note there is no detectable Cd or Co in samples if cells are not treated with these metals.



Vector Maps:

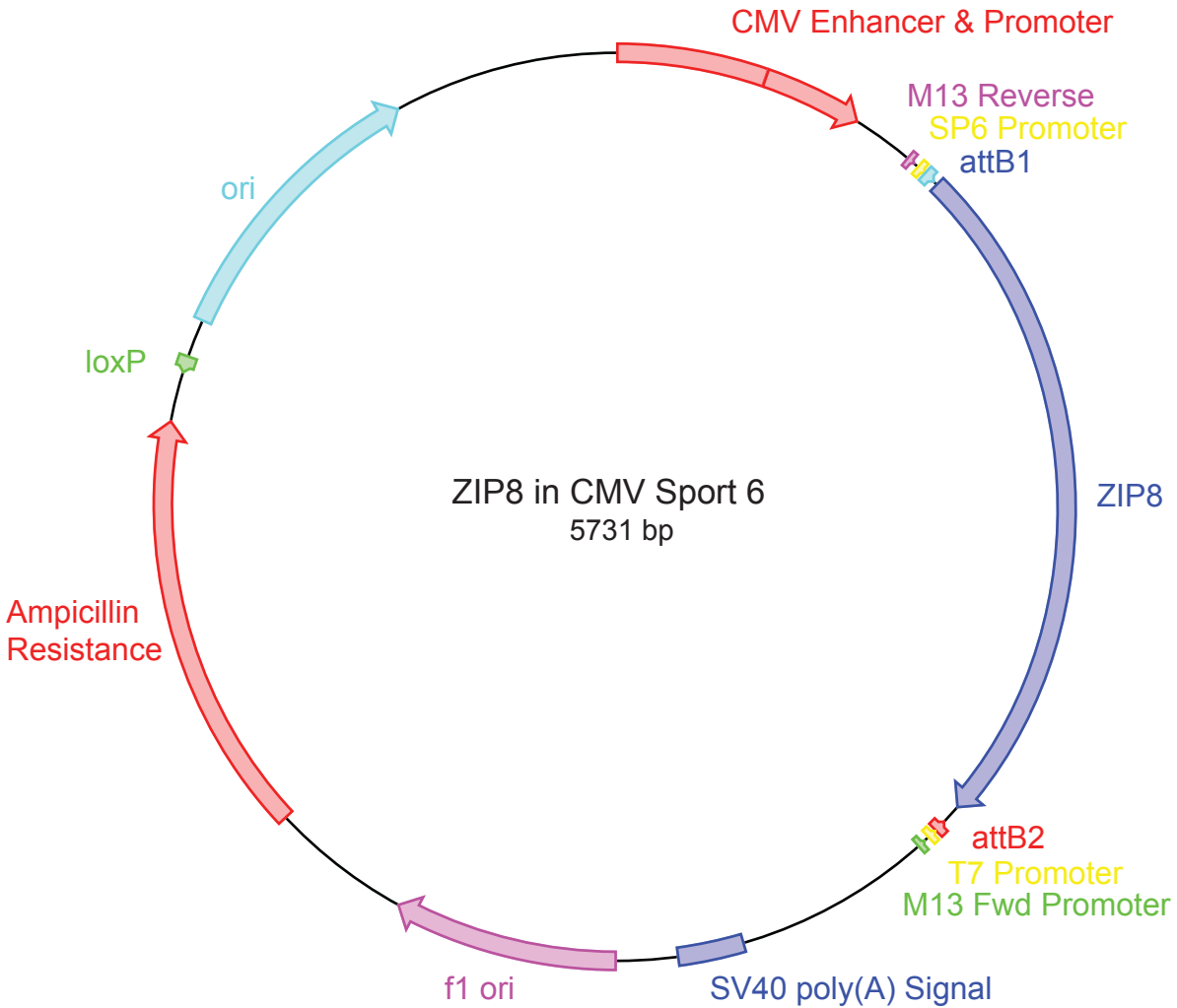
ZIP4 in CMV Sport6



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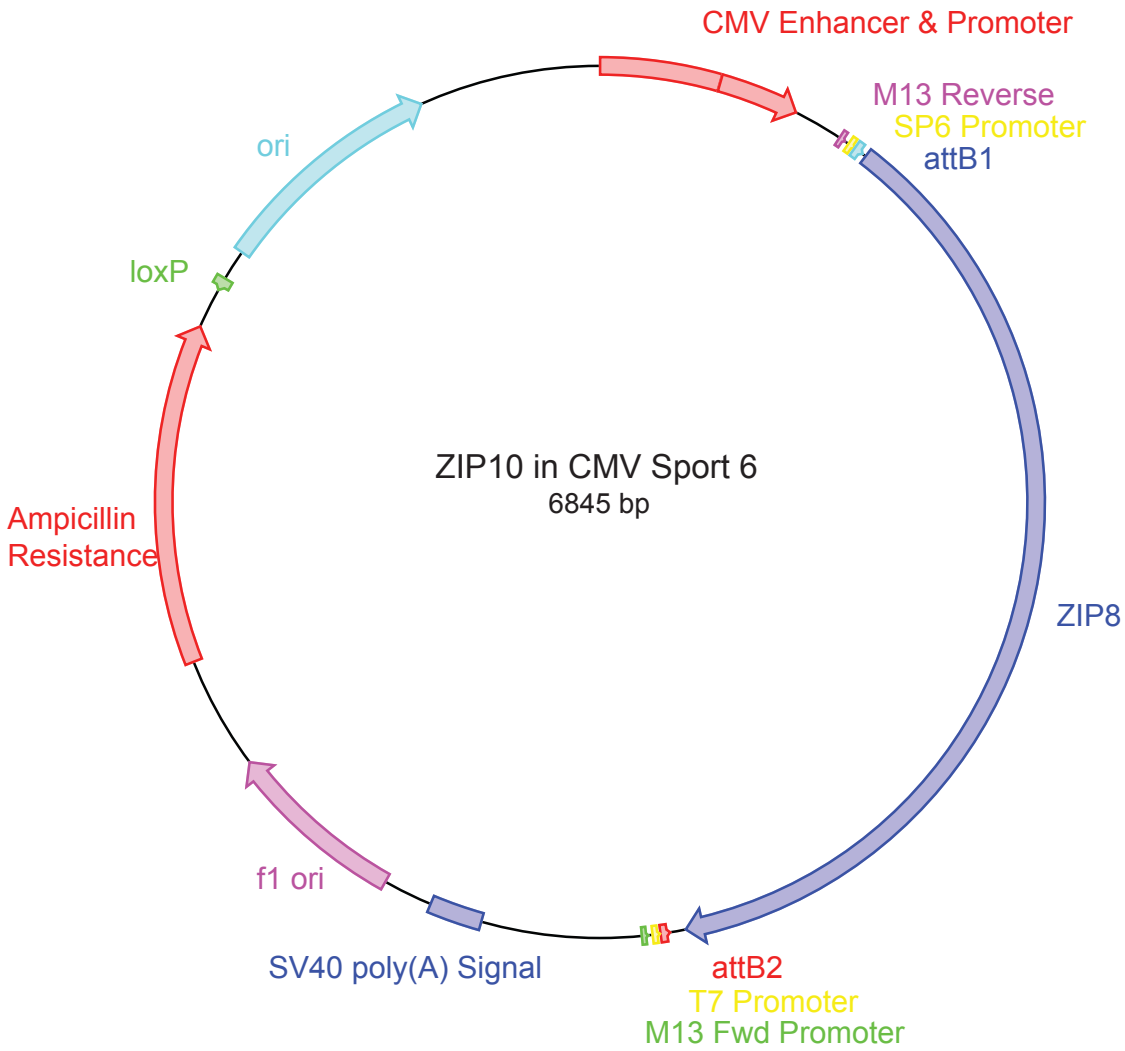
ZIP8  
Sport6

in CMV



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ZIP10 in CMV Sport6



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